NovaTec Immundiagnostica GmbH

NovaLisa®

Mycoplasma pneumoniae IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: MYCG0350 (96 Determinations)

ENGLISH

1. INTRODUCTION

The mycoplasms belong to the class Mollicutes comprising three distinct families and four genera, one of which is Mycoplasma with over 60 species. Mycoplasms are the smallest free living organisms known (300 to 500 nm in diameter) and unlike regular bacteria they lack a cell wall. Mycoplasms are extracellular parasites, especially on mucous membranes, which can cause infections in human, animals, plants, and cell cultures. Mycoplasma pneumoniae is primarily a respiratory pathogen (obligat) in human involving the nasopharynx, throat, trachea, bronchi, bronchioles, and alveoli. Other Mycoplasms, M. buccale, M. faucium, M. orale and M. salivarium are commensals in the oral cavity. Mycoplasma hominis and Ureaplasma urealyticum inhabit primarily the genital tract and may act as opportunistic invaders. M. pneumoniae is by far the most important pathogen of this group. Infection with M. pneumoniae occurs worldwide, its epidemiology has been studied primarily in the USA, Europe, and Japan. Infections are endemic in larger urban areas, and epidemic increases are observed at varying intervalls. M. pneumoniae has been estimated to cause 15-20% of all pneumoniae; the rate is highest in children and young adults. 74% of infections with M. pneumoniae are asymptomatic, reinfection may occur. Naturally acquired immunity to infection with M. pneumoniae appears to be of limited duration (2-3 years).

Species	Disease	Symptoms (e.g.)	Transmission route
M. pneumoniae	Respiratory diseases by Mycoplasma pneumoniae	Fever, headache, and a persistent cough. Respiratory tract disease: from asymptomatic infection to colds, pharyngitis, bronchitis, croup, tracheobronchitis, pneumonitis and primary atypical pneumonia	Transmitted by aerosol droplets

Infection or presence of pathogen may be identified by:

- Microscopy
- Serology: e.g. by ELISA

2. INTENDED USE

The Mycoplasma pneumoniae IgG ELISA is intended for the qualitative determination of IgG class antibodies against Mycoplasma pneumoniae in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Mycoplasma pneumoniae antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Mycoplasma pneumoniae antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection
IgA	Produced in mucosal linings throughout the body (⇒ protective barrier) Usually produced early in the course of the infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.434	3.80
#2	24	1.240	4.08
#3	24	1.635	6.03
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	32.58	5.73
#2	12	31.56	13.32
#3	12	4.36	11.10

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

It is 97.8% (95% confidence interval: 92.29% - 99.73%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

It is 100% (95% confidence interval: 96.87% - 100%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning		
	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: MYCG0350 Mycoplasma pneumoniae IgG ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Die Mykoplasmen gehören zur Klasse der Mollicutes, welche drei unterschiedliche Familien und vier Genera einschließt. Eines davon ist Mycoplasma mit über 60 Arten. Mycoplasmen sind die kleinsten bekannten frei lebenden Organismen (300 bis 500 nm Durchmesser). Die besitzen im Gegensatz zu Bakterien keine Zellwand. Mycoplasmen sind extrazelluläre Parasiten speziell der Schleimhäute und können bei Menschen, Tieren, Pflanzen und in Zellkulturen Infektionen hervorrufen. Mycoplasma pneumoniae ist in erster Linie ein obligat pathogener Erreger respiratorischer Erkrankungen unter Einbeziehung von Nasopharynx, Rachen, Luftröhre, Bronchien, Bronchiolen und Aveolen. Andere Mycoplasmen (M. buccale, M. faucium, M. orale, M. salivarium) sind Kommensalen in der Mundhöhle. Mycoplasma hominis und Ureaplasma urealyticum besiedeln vorwiegend den Genitaltrakt und können als opportunistische Invasoren auftreten. Mycoplasma pneumoniae ist mit Abstand das wichtigste Pathogen dieser Gruppe. Infektionen mit M. pneumoniae treten weltweit auf, deren Epidemiologie ist hauptsächlich in den USA, Europa und Japan untersucht worden. In größeren urbanen Regionen sind die Infektionen endemisch und epidemieartige Zuwächse werden in unregelmäßigen Abständen beobachtet. Es wird geschätzt, dass M. pneumoniae 15 bis 20 % aller Pneumonien verursacht; die Rate ist bei Kindern und jungen Erwachsenen am größten. 74 % der Infektionen mit M. pneumoniae verlaufen symptomlos; Reinfektionen können auftreten. Eine natürlich erworbene Immunität gegen Infektionen mit M. pneumoniae hält nur für 2 bis 3 Jahre an.

Spezies	**Erkrankung**	**Symptome (z.B.)**	**Infektionsweg**
M. pneumoniae	Erkrankungen der Atemwege duch Mycoplasma pneumoniae	Fieber, Kopfschmerzen und hartnäckiger Husten. Atemwegserkrankung, von asymptomatischer Infektion über Erkältungskrankheit, Pharyngitis, Bronchitis, Krupp, Tracheobronchitis, Pneumonitis und primäre atypische Pneumonie	Aerogen durch Tröpfchen

Nachweis des Erregers bzw. der Infektion durch:

- Mikroskopie
- Serologie: z.B. ELISA

2. VERWENDUNGSZWECK

Der Mycoplasma pneumoniae IgG ELISA ist für den qualitativen Nachweis spezifischer IgG-Antikörper gegen Mycoplasma pneumoniae in humanem Serum oder Plasma (Citrat, Heparin) bestimmt.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

4. MATERIALIEN

4.1. Mitgelieferte Reagenzien

- **Mikrotiterplatte:** 12 teilbare 8er-Streifen, beschichtet mit Mycoplasma pneumoniae Antigenen; in wieder verschließbarem Aluminiumbeutel.
- **IgG-Probenverdünnungspuffer:** 1 Flasche mit 100 mL Phosphatpuffer (10 mM) zur Probenverdünnung; pH 7,2 ± 0,2; gelb gefärbt; gebrauchsfertig; weiße Verschlusskappe; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Stopplösung:** 1 Flasche mit 15 mL Schwefelsäure, 0,2 mol/L; gebrauchsfertig; rote Verschlusskappe.
- **Waschpuffer (20x konz.):** 1 Flasche mit 50 mL eines 20-fach konzentrierten Phosphatpuffers (0,2 M), zum Waschen der Kavitäten; pH 7,2 ± 0,2; weiße Verschlusskappe.
- **Konjugat:** 1 Flasche mit 20 mL Peroxidase-konjugierten Antikörpern gegen humanes IgG in Phosphatpuffer (10 mM); blau gefärbt; gebrauchsfertig; schwarze Verschlusskappe.
- **TMB-Substratlösung:** 1 Flasche mit 15 mL 3,3`,5,5`-Tetramethylbenzidin (TMB), < 0,1 %; gebrauchsfertig; gelbe Verschlusskappe.
- **Positivkontrolle:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; rote Verschlusskappe; gebrauchsfertig; ≤ 0,02% (v/v) MIT.
- **Cut-off Kontrolle:** 1 Fläschchen mit 3 mL Kontrolle; gelb gefärbt; grüne Verschlusskappe; gebrauchsfertig; ≤ 0,02% (v/v) MIT.
- **Negativkontrolle:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; blaue Verschlusskappe; gebrauchsfertig; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Für Gefahren- und Sicherheitshinweise siehe 12.1.
Für potenzielle Gefahrstoffe überprüfen Sie bitte das Sicherheitsdatenblatt.

4.2. Mitgeliefertes Zubehör

- 1 selbstklebende Abdeckfolie
- 1 Arbeitsanleitung
- 1 Plattenlayout

4.3. Erforderliche Materialien und Geräte

- Mikrotiterplatten-Photometer mit Filtern 450/620 nm
- Inkubator 37 °C
- Manuelle oder automatische Waschvorrichtung für Mikrotiterplatten
- Mikropipetten (10 - 1000 µL)
- Vortex-Mischer
- Destilliertes Wasser
- Plastikröhrchen für den einmaligen Gebrauch

5. STABILITÄT UND LAGERUNG

Testkit bei 2...8 °C lagern. Die geöffneten Reagenzien sind bis zu den auf den Etiketten angegebenen Verfallsdaten verwendbar, wenn sie bei 2...8 °C gelagert werden.

6. VORBEREITUNG DER REAGENZIEN

Es ist sehr wichtig, alle Reagenzien und Proben vor ihrer Verwendung auf Raumtemperatur (20...25 °C) zu bringen und zu mischen!

6.1. Mikrotiterplatte

Die abbrechbaren Streifen sind mit Mycoplasma pneumoniae Antigenen beschichtet. Nicht verbrauchte Vertiefungen im Aluminiumbeutel zusammen mit dem Trockenmittel sofort wieder verschließen und bei 2...8 °C lagern.

6.2. Waschpuffer (20x konz.)

Der Waschpuffer ist im Verhältnis 1 + 19 zu verdünnen; z.B. 10 mL Waschpuffer + 190 mL destilliertes Wasser.
Der verdünnte Puffer ist bei Raumtemperatur (20…25 °C) 5 Tage haltbar. Sollten Kristalle im Konzentrat auftreten, die Lösung z.B. in einem Wasserbad auf 37 °C erwärmen und vor dem Verdünnen gut mischen.

6.3. TMB-Substratlösung

Die gebrauchsfertige Lösung ist bei 2...8 °C vor Licht geschützt aufzubewahren. Die Lösung ist farblos, kann aber auch leicht hellblau sein. Sollte die TMB-Substratlösung blau sein, ist sie kontaminiert und kann nicht im Test verwendet werden.

7. ENTNAHME UND VORBEREITUNG DER PROBEN

Es sollten humane Serum- oder Plasmaproben (Citrat, Heparin) verwendet werden. Werden die Bestimmungen innerhalb von 5 Tagen nach Blutentnahme durchgeführt, können die Proben bei 2...8 °C aufbewahrt werden, sonst aliquotieren und tiefgefrieren (-70…-20 °C). Wieder aufgetaute Proben vor dem Verdünnen gut schütteln. Wiederholtes Tiefgefrieren und Auftauen vermeiden!
Hitzeinaktivierung der Proben wird nicht empfohlen.

7.1. Probenverdünnung

Proben vor Testbeginn im Verhältnis 1 + 100 mit IgG-Probenverdünnungspuffer verdünnen, z. B. 10 µL Probe und 1 mL IgG-Probenverdünnungspuffer in die entsprechenden Röhrchen pipettieren, um eine Verdünnung von 1 + 100 zu erhalten; gut mischen (Vortex).

8. TESTDURCHFÜHRUNG

Arbeitsanleitung **vor** Durchführung des Tests sorgfältig lesen. Für die Zuverlässigkeit der Ergebnisse ist es notwendig, die Arbeitsanleitung genau zu befolgen. Die folgende Testdurchführung ist für die manuelle Methode validiert. Beim Arbeiten mit ELISA Automaten empfehlen wir, um Wascheffekte auszuschließen, die Zahl der Waschschritte von drei auf bis zu fünf und das Volumen des Waschpuffers von 300 µL auf 350 µL zu erhöhen. Kapitel 12 beachten. Vor Testbeginn auf dem mitgelieferten Plattenlayout die Verteilung bzw. Position der Proben und der Standards/Kontrollen (Doppelbestimmung empfohlen) genau festlegen. Die benötigte Anzahl von Mikrotiterstreifen (Kavitäten) in den Streifenhalter einsetzen.

Den Test in der angegebenen Reihenfolge und ohne Verzögerung durchführen.

Für jeden Pipettierschritt der Standards/Kontrollen und Proben saubere Einmalspitzen verwenden.

Den Inkubator auf 37 ± 1 °C einstellen.

1. Je 100 µL Standards/Kontrollen und vorverdünnte Proben in die entsprechenden Vertiefungen pipettieren. Vertiefung A1 ist für den Substratleerwert vorgesehen.
2. Die Streifen mit der mitgelieferten Abdeckfolie bedecken.
3. **1 h ± 5 min bei 37 ± 1 °C inkubieren.**
4. Am Ende der Inkubationszeit Abdeckfolie entfernen und die Inkubationsflüssigkeit aus den Teststreifen absaugen. Anschließend dreimal mit 300 µL Waschpuffer waschen. Überfließen von Flüssigkeit aus den Vertiefungen vermeiden. Das Intervall zwischen Waschen und Absaugen sollte > 5 sec betragen. Nach dem Waschen die Teststreifen auf Fließpapier ausklopfen, um die restliche Flüssigkeit zu entfernen.

   Beachte: Der Waschvorgang ist wichtig, da unzureichendes Waschen zu schlechter Präzision und falschen Messergebnissen führt!
5. 100 µL Konjugat in alle Vertiefungen, mit Ausnahme der für die Berechnung des Leerwertes A1 vorgesehenen, pipettieren.
6. **30 min bei Raumtemperatur (20...25 °C) inkubieren.** Nicht dem direkten Sonnenlicht aussetzen.
7. Waschvorgang gemäß Punkt 4 wiederholen.
8. 100 µL TMB-Substratlösung in alle Vertiefungen pipettieren.
9. **Genau 15 min im Dunkeln bei Raumtemperatur (20...25 °C) inkubieren.** Bei enzymatischer Reaktion findet eine Blaufärbung statt.
10. In alle Vertiefungen 100 µL Stopplösung in der gleichen Reihenfolge und mit den gleichen Zeitintervallen wie bei Zugabe der TMB-Substratlösung pipettieren, dadurch erfolgt ein Farbwechsel von blau nach gelb.
11. Die Extinktion der Lösung in jeder Vertiefung bei 450/620 nm innerhalb von 30 min nach Zugabe der Stopplösung messen.

8.1. Messung

Mit Hilfe des Substratleerwertes den **Nullabgleich** des Mikrotiterplatten-Photometers vornehmen.

Falls diese Eichung aus technischen Gründen nicht möglich ist, muss nach der Messung der Extinktionswert des Substratleerwertes von allen anderen Extinktionswerten subtrahiert werden, um einwandfreie Ergebnisse zu erzielen!

Extinktion aller Kavitäten bei **450 nm** messen und die Messwerte der Standards/Kontrollen und Proben in das Plattenlayout eintragen.

Eine **bichromatische** Messung mit der Referenzwellenlänge 620 nm wird empfohlen.

Falls Doppel- oder Mehrfachbestimmungen durchgeführt wurden, den **Mittelwert der Extinktionswerte** berechnen.

9. BERECHNUNG DER ERGEBNISSE

9.1. Testgültigkeitskriterien

Damit ein Testlauf als valide betrachtet werden kann, muss diese Gebrauchsanweisung strikt befolgt werden, und die folgenden Kriterien müssen erfüllt sein:

- **Substrat-Leerwert**: Extinktionswert **< 0,100**
- **Negativkontrolle**: Extinktionswert **< 0,200 und < Cut-off**
- **Cut-off Kontrolle**: Extinktionswert **0,150 – 1,300**
- **Positivkontrolle**: Extinktionswert **> Cut-off**

Sind diese Kriterien nicht erfüllt, ist der Testlauf ungültig und muss wiederholt werden.

9.2. Messwertberechnung

Der Cut-off ergibt sich aus dem Mittelwert der gemessenen Extinktionen der Cut-off Kontrolle.

Beispiel: 0,44 OD Cut-off Kontrolle + 0,42 OD Cut-off Kontrolle = 0,86: 2 = 0,43
Cut-off = 0,43

9.2.1. Ergebnisse in Einheiten [NTU]

$$\frac{\text{Mittlere Extinktion der Probe} \times 10}{\text{Cut-off}} = \text{[NovaTec Einheiten = NTU]}$$

Beispiel: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretation der Ergebnisse

Cut-off	10 NTU	-
Positiv	> 11 NTU	Es liegen Antikörper gegen den Erreger vor. Ein Kontakt mit dem Antigen (Erreger bzw. Impfstoff) hat stattgefunden.
Grenzwertig	9 – 11 NTU	Antikörper gegen den Erreger können nicht eindeutig nachgewiesen werden. Es wird empfohlen den Test nach 2 bis 4 Wochen mit einer frischen Patientenprobe zu wiederholen. Finden sich die Ergebnisse erneut im grenzwertigen Bereich, gilt die Probe als **negativ**.
Negativ	< 9 NTU	Es liegen keine Antikörper gegen den Erreger vor. Ein vorausgegangener Kontakt mit dem Antigen (Erreger bzw. Impfstoff) ist unwahrscheinlich.
Die Diagnose einer Infektionskrankheit darf nicht allein auf der Basis des Ergebnisses einer Bestimmung gestellt werden. Die anamnestischen Daten sowie die Symptomatologie des Patienten müssen zusätzlich zu den serologischen Ergebnissen in Betracht gezogen werden. Bei Immunsupprimierten und Neugeborenen besitzen die Ergebnisse serologischer Tests nur einen begrenzten Wert.		

9.3.1. Antikörper-Isotypen und Infektionsstatus

Serologie	Bedeutung
IgM	Typisch für Primärantwort Hoher IgM-Titer bei gleichzeitig niedrigem IgG-Titer: → Hinweis auf relativ frische Infektion Selten: → persistierendes IgM
IgG	Typisch für Sekundärantwort Können auch noch nach Jahren nachweisbar sein Hoher IgG-Titer bei gleichzeitig niedrigem IgM-Titer: → wahrscheinlich länger zurückliegende Infektion
IgA	Sezerniert in allen Schleimhäuten (⇒ Schutzbarriere) Meist früh im Verlauf einer Infektion gebildet

10. TESTMERKMALE

Die Ergebnisse beziehen sich auf die untersuchten Probenkollektive; es handelt sich nicht um garantierte Spezifikationen.
Für weitere Informationen zu den Testmerkmalen kontaktieren Sie bitte NovaTec Immundiagnostica GmbH.

10.1. Präzision

Intraassay	**n**	**Mittelwert (E)**	**CV (%)**
#1	24	0,434	3,80
#2	24	1,240	4,08
#3	24	1,635	6,03
Interassay	**n**	**Mittelwert (NTU)**	**CV (%)**
#1	12	32,58	5,73
#2	12	31,56	13,32
#3	12	4,36	11,10

10.2. Diagnostische Spezifität

Die diagnostische Spezifität ist definiert als die Wahrscheinlichkeit des Tests, ein negatives Ergebnis bei Fehlen des spezifischen Analyten zu liefern. Sie beträgt 97,8% (95% Konfidenzintervall: 92,29% - 99,73%).

10.3. Diagnostische Sensitivität

Die diagnostische Sensitivität ist definiert als die Wahrscheinlichkeit des Tests, ein positives Ergebnis bei Vorhandensein des spezifischen Analyten zu liefern. Sie ist 100% (95% Konfidenzintervall: 96,87% - 100%).

10.4. Interferenzen

Hämolytische, lipämische und ikterische Proben ergaben bis zu einer Konzentration von 10 mg/mL Hämoglobin, 5 mg/mL Triglyceride und 0,5 mg/mL Bilirubin keine Interferenzen im vorliegenden ELISA.

10.5. Kreuzreaktivität

Die Untersuchung eines Probenpanels mit Antikörperaktivitäten gegen potenziell kreuzreagierende Parameter ließ keine Anzeichen von falsch-positiven Ergebnissen aufgrund von Kreuzreaktivitäten erkennen.

11. GRENZEN DES VERFAHRENS

Kontamination der Proben durch Bakterien oder wiederholtes Einfrieren und Auftauen können zu einer Veränderung der Messwerte führen.

12. SICHERHEITSMASSNAHMEN UND WARNHINWEISE

- Die Testdurchführung, die Information, die Sicherheitsmaßnahmen und Warnhinweise in der Arbeitsanleitung sind strikt zu befolgen. Bei Anwendung des Testkits auf Diagnostika-Geräten ist die Testmethode zu validieren. Jede Änderung am Aussehen, der Zusammensetzung und der Testdurchführung sowie jede Verwendung in Kombination mit anderen Produkten, die der Hersteller nicht autorisiert hat, ist nicht zulässig; der Anwender ist für solche Änderungen selbst verantwortlich. Der Hersteller haftet für falsche Ergebnisse und Vorkommnisse aus solchen Gründen nicht. Auch für falsche Ergebnisse aufgrund von visueller Auswertung wird keine Haftung übernommen.
- Nur für in-vitro-Diagnostik.
- Alle Materialien menschlichen oder tierischen Ursprungs sind als potentiell infektiös anzusehen und entsprechend zu behandeln.
- Alle verwendeten Bestandteile menschlichen Ursprungs sind auf Anti-HIV-AK, Anti-HCV-AK und HBsAg nicht-reaktiv getestet.
- Reagenzien und Mikrotiterplatten unterschiedlicher Chargen nicht untereinander austauschen.
- Keine Reagenzien anderer Hersteller zusammen mit den Reagenzien dieses Testkits verwenden.
- Nicht nach Ablauf des Verfallsdatums verwenden.
- Nur saubere Pipettenspitzen, Dispenser und Labormaterialien verwenden.
- Verschlusskappen der einzelnen Reagenzien nicht untereinander vertauschen, um Kreuzkontaminationen zu vermeiden.
- Flaschen sofort nach Gebrauch fest verschließen, um Verdunstung und mikrobielle Kontamination zu vermeiden.
- Nach dem ersten Öffnen Konjugat und Standards/Kontrollen vor weiterem Gebrauch auf mikrobielle Kontamination prüfen.
- Zur Vermeidung von Kreuzkontamination und falsch erhöhten Resultaten, Reagenzien sorgfältig in die Kavitäten pipettieren.
- Der ELISA ist nur für qualifiziertes Personal bestimmt, das den Standards der Guten Laborpraxis (GLP) folgt.
- Zur weiteren internen Qualitätskontrolle sollte jedes Labor zusätzlich bekannte Proben verwenden.

12.1. Sicherheitshinweis für Reagenzien, die Gefahrstoffe enthalten

Die Reagenzien können CMIT/MIT (3:1) oder MIT enthalten (siehe 4.1)
Daher gelten die folgenden Gefahren- und Sicherheitshinweise.

Achtung

H317	Kann allergische Hautreaktionen verursachen.
P261	Einatmen von Aerosol vermeiden.
P280	Schutzhandschuhe/ Schutzkleidung tragen.
P302+P352	BEI BERÜHRUNG MIT DER HAUT: Mit viel Seife und Wasser waschen.
P333+P313	Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen/ ärztliche Hilfe hinzuziehen.
P362+P364	Kontaminierte Kleidung ausziehen und vor erneutem Tragen waschen

Weitere Informationen können dem Sicherheitsdatenblatt entnommen werden.

12.2. Entsorgungshinweise

Chemikalien und Zubereitungen sind in der Regel Sonderabfälle. Deren Beseitigung unterliegt den nationalen abfallrechtlichen Gesetzen und Verordnungen. Die zuständige Behörde informiert über die Entsorgung von Sonderabfällen.

13. BESTELLINFORMATIONEN

Produktnummer: MYCG0350 Mycoplasma pneumoniae IgG ELISA (96 Bestimmungen)

FRANÇAIS

1. INTRODUCTION

Les mycoplasmes appartiennent à la classe des Mollicutes qui comprend trois familles distinctes et quatre genres, dont l'un est le mycoplasme avec plus de 60 espèces. Les Mycoplasmes sont les plus petits organismes libres connus (300 à 500 nm de diamètre) et contrairement aux bactéries régulières ils n'ont pas de membrane cellulaire. Les mycoplasmes sont des parasites extracellulaires, particulièrement sur les membranes muqueuses, qui peuvent causer des infections dans l'homme, les animaux, les plantes, et les cultures cellulaires. Mycoplasma pneumoniae est d'abord un pathogène respiratoire (obligatoire) dans l'homme qui peut atteindre le nasopharynx, la gorge, la trachée, les bronches, les bronchioles, et les alvéoles. D'autres mycoplasmes, M. buccale, M. faucium, M. orale et M. salivarium sont des commensales dans la cavité buccale. Mycoplasma hominis et Ureaplasma urealyticum habitent surtout la région génitale et peuvent agir en tant qu'envahisseurs opportunistes. Mycoplasma pneumoniae est de loin le microbe pathogène le plus important de ce groupe. L'infection avec Mycoplasma pneumoniae se produit dans le monde entier, son épidémiologie a surtout été étudiée aux Etats-Unis, en Europe, et au Japon. Les infections sont endémiques dans les grands secteurs urbains, et des augmentations épidémiques sont observées à intervalles variables. On a estimé que Mycoplasma pneumoniae cause 15-20% de toutes les pneumonies; le taux est le plus élevé chez les enfants et de jeunes adultes. 74% des infections avec Mycoplasma pneumoniae sont asymptomatiques, et des réinfections peuvent se produire. L'immunité naturellement acquise contre l'infection avec Mycoplasma pneumoniae semble être de durée limitée (2 ou 3 ans).

Espèce	**La maladie**	**Symptômes (p.ex.)**	**Modes de transmission**
Mycoplasma pneumoniae	les maladies de région respiratoire par Mycoplasma pneumoniae	Fièvre, maux de tête et toux persistante. Maladie de région respiratoire: de l'infection asymptomatique à la pharyngite, la bronchite, le croup, la trachéo-bronchite et à la pneumonie atypique primaire	La transmission du virus se produit principalement par le contact de gouttelettes.

L'infection ou la présence d'un agent pathogène peut être identifiée par:

- Microscopie
- Sérologie: p.ex. ELISA

2. INDICATION D'UTILISATION

La trousse Mycoplasma pneumoniae IgG ELISA est prévue pour la détection qualitative des anticorps IgG anti-Mycoplasma pneumoniae dans le sérum humain ou plasma (citrate, héparine).

3. PRINCIPE DU TEST

La détermination immunoenzymatique qualitative des anticorps spécifiques est basée sur le la technique ELISA (du anglais, Enzyme-Linked Immunosorbent Assay).
Plaques de Microtitrage sont recouvertes d'antigènes spécifiques pour lier les anticorps correspondants de l'échantillon. Après le lavage des puits pour éliminer l'échantillon détaché, le conjugué peroxydase de raifort (HRP) est ajouté. Ce conjugué se lie aux anticorps capturés. Dans une deuxième étape de lavage, le conjugué non lié est éliminé. Le complexe immun formé par le conjugué lié est visualisé par l'addition tétraméthylbenzidine (TMB) qui donne un produit de réaction bleu.
L'intensité de ce produit est proportionnelle à la quantité d'anticorps spécifiques dans l'échantillon. L'acide sulfurique est ajouté pour arrêter la réaction. Cela produit un changement du bleu au jaune. L'absorbance à 450/620 nm est lue en utilisant un Photomètre de Plaque de Microtitrage ELISA.

4. MATERIEL

4.1. Réactifs fournis

- **Plaque de Microtitrage:** 12 barrettes de 8 puits sécables revêtus d'antigène d'Mycoplasma pneumoniae; en sachets d'aluminium refermables.
- **Tampon de Dilution d'Échantillon IgG:** 1 flacon contenant 100 mL de tampon phosphaté (10 mM) pour la dilution de l'échantillon; pH 7,2 ± 0,2; prêt à l'emploi; couleur jaune; bouchon blanc; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solution d'arrêt:** 1 flacon contenant 15 mL d'acide sulfurique, 0,2 mol/L; prêt à l'emploi; bouchon rouge.
- **Tampon de Lavage (concentré x 20):** 1 flacon contenant 50 mL d'un tampon phosphaté (0,2 M) concentré 20 fois (pH 7,2 ± 0,2) pour laver les puits; bouchon blanc.
- **Conjugué:** 1 flacon contenant 20 mL d'anticorps IgG anti-humaines conjuguées à de la peroxydase du raifort dans le tampon phosphaté (10 mM); prêt à l'emploi; couleur bleue, bouchon noir.
- **Solution de Substrat TMB:** 1 flacon contenant 15 mL de 3,3',5,5'-tétraméthylbenzidine (TMB), < 0,1 %; prêt à l'emploi; bouchon jaune.
- **Contrôle Positif:** 1 flacon contenant 2 mL contrôle; prêt à l'emploi; couleur jaune; bouchon rouge; ≤ 0,02% (v/v) MIT.
- **Contrôle Cut-off:** 1 flacon contenant 3 mL contrôle; prêt à l'emploi; couleur jaune; bouchon vert; ≤ 0,02% (v/v) MIT.
- **Contrôle Négatif:** 1 flacon contenant 2 mL contrôle; prêt à l'emploi; couleur jaune; bouchon bleu; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Pour les mentions de danger et les conseils de prudence voir chapitre 12.1.
Pour les substances potentiellement dangereuses s'il vous plaît vérifiez la fiche de données de sécurité.

4.2. Matériel fourni

- 1 couvercle autocollante
- 1 instructions d'utilisation
- 1 présentation de la plaque

4.3. Matériel et équipement requis

- Photomètre de Plaque de Microtitrage ELISA, pour mesurer l'absorbance à 450/620 nm
- Incubateur 37 °C
- Laveur manuel ou automatique pour le lavage des Plaques de Microtitrage
- Pipettes pour utilisation entre 10 et 1000 µL
- Mélangeur Vortex
- Eau distillée
- Tubes jetables

5. STABILITE ET CONSERVATION

Conserver le kit à 2...8 °C. Les réactifs ouverts sont stables jusqu'à la date de péremption indiquée sur l'étiquette lorsqu'il est conservé à 2...8 °C.

6. PREPARATION DES REACTIFS

Il est très important porter tous les réactifs et échantillons à température ambiante (20... 25 °C) et les mélanger avant de commencer le test!

6.1. Plaque de Microtitrage

Les barrettes sécables sont revêtues d'antigène d'Mycoplasma pneumoniae. Immédiatement après avoir prélevé les barrettes nécessaires, les barrette restantes doivent être scellés le vide dans de feuille d'aluminium avec le sac de silicium (le déshydratant) fourni et emmagasiner à 2...8 °C.

6.2. Tampon de Lavage (conc. x 20)

Diluer le Tampon de Lavage 1+19; par exemple 10 mL du Tampon de Lavage + 190 mL d'eau distillée. Le Tampon de Lavage diluée est stable pendant 5 jours à la température ambiante (20…25 °C). Cas apparaissent des cristaux dans le concentré, chauffer la solution à 37 °C par exemple dans un bain-marie mélangez bien avant dilution.

6.3. Solution de Substrat TMB

La solution est prête à utiliser et doit être emmagasiné à 2...8 °C, à l'abri de la lumière. La solution doit être incolore ou pourrait avoir une légère couleur bleu clair. Si le substrat devient bleu, il peut avoir été contaminé et ne peut pas être utilisé dans le test.

7. PRELEVEMENT ET PREPARATION DES ECHANTILLONS

Utiliser des échantillons humains de sérum ou plasma (citrate, héparine) pour ce test. Si le test est réalisé dans les 5 jours après le prélèvement, les échantillons doivent être conservés à 2...8 °C; autrement ils doivent être aliquotés et conservés surgelés (-70…-20 °C). Si les échantillons sont conservés congelés, bien mélanger les échantillons décongelés avant le test. Éviter les cycles répétés de congélation et décongélation.
L'inactivation par la chaleur des échantillons n'est pas recommandée.

7.1. Dilution de l'échantillon

Avant du test, tous les échantillons doivent être dilués 1 + 100 avec Tampon de Dilution d'Échantillon IgG. Diluer 10 µL d'échantillon avec 1 mL I Tampon de Dilution d'Échantillon IgG dans des tubes pour obtenir une dilution 1 + 100 et mélanger soigneusement sur un Vortex.

8. PROCEDE DE TEST

Lire attentivement les instructions d'utilisation **avant de** réaliser le test. La fiabilité des résultats dépend du suivi strict d'utilisation comme décrit. La technique de test suivante a été validée uniquement pour une procédure manuelle. Si le test doit être effectué sur un systèmes automatiques pour ELISA, nous conseillons d'augmenter le nombre d'étapes de lavage de trois à cinq et le volume du Tampon de Lavage de 300 à 350 µL. Faites attention au chapitre 12. Avant de commencer le test, le plan de distribution et d'identification de tous les échantillons et les étalons/contrôles (il est recommandé déterminer en double) doivent être soigneusement établi sur la feuille présentation de la plaque prévue dans le conseil de kit. Sélectionner le nombre de barrettes ou de puits nécessaires et les placer sur le support.

Réaliser toutes les étapes du test dans l'ordre donné et sans délai.

Un embout de pipette propre et jetable doit être utilisé pour distribuer chaque étalon/contrôle et échantillon.

Régler l'incubateur à 37 ± 1 °C.

1. Pipeter 100 µL de étalons/contrôles et d'échantillons dilués dans leurs puits respectifs. Garder le puits A1 pour le blanc substrat.
2. Couvrir les puits avec le couvercle, fourni dans le kit.
3. **Incuber pendant 1 heure ± 5 minutes à 37 ± 1 °C.**
4. A la fin de l'incubation, enlever le couvercle, aspirer le contenu des puits et laver chaque puits trois fois avec 300 µL du Tampon de Lavage. Éviter les débordements des puits de réaction. L'intervalle entre le cycle de lavage et l'aspiration doit être > 5 sec. À la fin, enlever soigneusement le liquide restant en tapotant les barrettes sur du papier absorbant avant la prochaine étape.

   Note: L'étape de lavage est très importante! Un lavage insuffisant peut conduire à une précision faible et de faux résultats !
5. Pipeter 100 µL du conjugué dans tous les puits sauf le puits Blanc A1.
6. **Incuber pendant 30 minutes à température ambiante (20…25°C).** N'exposer pas à la lumière directe du soleil.
7. Répéter l'étape numéro 4.
8. Pipeter 100 µL de la Solution de Substrat TMB dans tous les puits.
9. **Incuber pendant exactement 15 minutes à température ambiante (20…25°C) dans l'obscurité.** Une couleur bleue se produit en raison d'une réaction enzymatique.
10. Pipeter 100 µL de la solution d'arrêt dans tous les puits dans le même ordre et à la même vitesse que pour la Solution de Substrat TMB, ainsi, il y a un changement du bleu au jaune.
11. Mesurer l'absorbance à 450/620 nm dans les 30 minutes après l'addition de la solution d'arrêt.

8.1. Mesure

Réglez le Photomètre de Plaque de Microtitrage ELISA **à zéro** en utilisant **le Blanc substrat.**
Si - pour des raisons techniques - le Photomètre de Plaque de Microtitrages ELISA ne peut pas être ajusté à zéro en utilisant le Blanc substrat, la valeur d'absorbance de cette doit être soustraire la valeur d'absorbance de toutes les autres valeurs d'absorbance mesurées afin d'obtenir des résultats fiables!

Mesurer l'absorbance de tous les puits **à 450 nm** et enregistrer les valeurs d'absorbance pour chaque étalon/contrôle et échantillon dans la présentation de la plaque.

Il est recommandé d'effectuer la mesure **dichromatique** utilisant 620 nm comme longueur d'onde de référence.
Si doubles déterminations ont été effectuées, calculer **les valeurs moyennes d'absorbance**.

9. RESULTATS

9.1. Critères de validation

Pour qu'une série d'analyses soit considérée comme valide, ces instructions d'utilisation doivent être strictement suivies, et les critères suivants doivent être respectés:

- **Blanc Substrat**: Valeur d'absorbance **< 0,100**
- **Contrôle Négatif**: Valeur d'absorbance **< 0,200 et < Cut-off**
- **Contrôle Cut-off**: Valeur d'absorbance **0,150 – 1,300**
- **Contrôle Positif**: Valeur d'absorbance **> Contrôle Cut-off**

Lorsque ces critères ne sont pas remplis, le test n'est pas valide et doit être recommencé.

9.2. Calcul des résultats

La valeur seuil correspond à la moyenne des valeurs d'absorbance du Contrôle Cut-off.

Exemple: 0,44 DO Contrôle Cut-off + 0,42 DO Contrôle Cut-off = 0,86: 2 = 0,43

Cut-off = 0,43

9.2.1. Résultats en unités [NTU]

$$\frac{\text{Valeur (moyenne) d'absorbance de l' échantillon} \times 10}{\text{Cut-off}} = \text{[unités NovaTec = NTU]}$$

Exemple: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interprétation des résultats

Cut-off	10 NTU	-
Positif	> 11 NTU	Les anticorps dirigés contre l'agent pathogène sont présents. Il ya eu un contact avec l'antigène (pathogène resp. vaccin).
Zone grise	9 – 11 NTU	Les anticorps dirigés contre l'agent pathogène ne pouvaient pas être détectés clairement. Il est recommandé de répéter le test avec un échantillon frais dans 2 à 4 semaines. Si le résultat est encore dans la zone grise l'échantillon est jugé **négatif**.
Negatif	< 9 NTU	L'échantillon ne contient pas d'anticorps contre l'agent pathogène. Un contact préalable avec l'antigène (pathogène resp. vaccin) est peu probable.
Le diagnostic d'une maladie infectieuse ne devrait pas être établi sur la base du résultat d'une seule analyse. Un diagnostic précis devrait prendre en considération l'histoire clinique, la symptomatologie ainsi que les données sérologiques. Les données sérologiques sont de valeur limité dans le cas des patients immunodéprimés et des nouveaux-nés.		

9.3.1. Isotypes d'anticorps et l'Etat de l'infection

Sérologie	Signification
IgM	Caractéristique de la réponse primaire du anticorps Titre élevé d'IgM avec une faible titre d'IgG: → suggère une infection très récente ou aigüe Rare: → persistante IgM
IgG	Caractéristique de la réponse secondaire du anticorps Peut persister pendant plusieurs années Des titres élevés d'IgG à faible titre d'IgM: → peuvent indiquer une infection ancienne
IgA	Ils sont produits au niveau des muqueuses dans tout le corps (⇒ barrière de protection) Habituellement ils sont produits en début d'infection

10. PERFORMANCES DU TEST

Ces résultats s'appuient sur les groupes d'échantillons étudiés; il n'agit pas de caractéristiques techniques garanties.

Pour plus d'informations sur les performances du test s'il vous plaît contactez NovaTec Immundiagnostica GmbH.

10.1. Précision

Intra-essai	**n**	**moyenne (DO)**	**CV (%)**
#1	24	0,434	3,80
#2	24	1,240	4,08
#3	24	1,635	6,03
Inter-essai	**n**	**moyenne (NTU)**	**CV (%)**
#1	12	32,58	5,73
#2	12	31,56	13,32
#3	12	4,36	11,10

10.2. Spécificité diagnostique

La spécificité diagnostique est définie comme la probabilité d'obtenir un résultat négatif en l'absence d'un analyte spécifique. Elle est 97,8% (95% Intervalle de confiance: 92,29% - 99,73%).

10.3. Sensibilité diagnostique

La sensibilité diagnostique est définie comme la probabilité d'obtenir un résultat positif en présence d'un analyte spécifique. Elle est 100% (95% Intervalle de confiance: 96,87% - 100%).

10.4. Interférences

Des échantillons hémolytiques ou lipémiques ou ictériques n'ont pas montré d'interférences, avec des concentrations jusqu'à 10 mg/mL de hémoglobine, 5 mg/mL de triglycérides et 0,5 mg/mL de bilirubine.

10.5. Réaction croisée

L'étude d'un panel d'échantillons avec des anticorps dirigés contre différents paramètres interférents n'a pas révélé de preuves de résultats faussement positifs dus à des réactions croisées.

11. LIMITES DE LA TECHNIQUE

Une contamination bactérienne ou des cycles de congélation/décongélation répétés de l´échantillon peuvent affecter les valeurs d'absorption.

12. PRECAUTIONS ET AVERTISSEMENTS

- La procédure de test, l'information, les précautions et mises en garde de la notice d'emploi, doivent être suivies de façon stricte. L'utilisation de ces trousses avec des automates ou dispositifs similaires doit être validée. Aucun changement de la conception, composition et procédure de test, ainsi que l'utilisation avec d'autres produits non approuvés par le fabricant, ne sont pas autorisés; seul l'utilisateur est responsable de tels changements. Le fabricant n'est pas responsable des faux résultats et des incidents dus à ces motifs. Le fabricant n'est pas responsable des résultats fournis par analyse visuelle des échantillons des patients.
- Uniquement pour diagnostic in vitro.
- Tous les matériaux d'origine humaine ou animale doivent être considérés et traités comme étant potentiellement infectieux.
- Tous les composants d'origine humaine utilisés pour la fabrication de ces réactifs ont été analysés et ont été trouvés non réactifs en Ag HBs, en anticorps anti-VHI 1 et 2 et en anticorps anti-VHC.
- Ne pas échanger les réactifs ou les Plaque de Microtitrage provenant de différents lots de production.
- Ne pas utiliser de réactifs provenant d'autres fabricants avec les réactifs de cette trousse.
- Ne pas utiliser les réactifs après la date de péremption indiquée sur l'étiquette.
- Utiliser seulement des embouts de pipette, des distributeurs et du matériel de laboratoire propres.
- Ne pas échanger les bouchons des flacons, pour éviter la contamination croisée.
- Fermer soigneusement les flacons après utilisation pour éviter l'évaporation et la contamination microbienne.
- Avant une nouvelle utilisation, vérifier les flacons de conjugué et de étalon/contrôle, déjà utilisés, pour exclure une contamination microbienne.
- Pour éviter la contamination croisée et des résultats faussement élevés, introduire les échantillons de patients et les réactifs exactement au fond des puits sans éclabousser.
- L'ELISA est uniquement conçu pour le personnel qualifié suivant les normes de bonnes pratiques de laboratoire (Good Laboratory Practice, GLP).
- Pour un contrôle de qualité interne plus poussé, chaque laboratoire doit en outre utiliser des échantillons connus.

12.1. Note de sécurité pour les réactifs contenant des substances dangereuses

Les réactifs peuvent contenir du CMIT/MIT (3 :1) ou du MIT (voir chapitre 4.1)
Par conséquent, les mentions de danger et les conseils de prudence suivants s'appliquent.

Attenttion

H317	Peut provoquer une allergie cutanée
P261	Éviter de respirer les aérosols
P280	Porter des gants de protection/ des vêtements de protection.
P302+P352	EN CAS DE CONTACT AVEC LA PEAU: Laver abondamment savon à l'eau.
P333+P313	En cas d'irritation ou d'éruption cutanée: consulter un médecin.
P362+P364	Enlever les vêtements contaminés et les laver avant réutilisation.

De plus amples informations peuvent être trouvées dans la fiche de données de sécurité.

12.2. Elimination des déchets

Les résidus des produits chimiques et des préparations sont considérés en général comme des déchets dangereux. L'élimination de ce type de déchet est réglementée par des lois et réglementations nationales et régionales. Contacter les autorités compétentes ou les sociétés de gestion des déchets pour obtenir des renseignements sur l'élimination des déchets dangereux.

13. INFORMATION POUR LES COMMANDES

Référence:	MYCG0350	Mycoplasma pneumoniae IgG ELISA (96 déterminations)

ITALIANO

1. INTRODUZIONE

Il Mycoplasma pneumoniae è un membro della classe dei Mollicuti, in quanto privo di parete cellulare e rivestito soltanto da membrana plasmatica. Pertanto rappresenta la più piccola cellula vivente al momento conosciuta. La famiglia dei Mycoplasmataceae si divide nei generi Mycoplasma ed Ureaplasma. Al momento sono conosciute più di 80 specie. Le specie più importanti e patogene per l'uomo sono: M. pneumonite, M. hominis e M. genitalis. Oltre a queste esistono altre specie eventualmente patogene: M. orale, M. salivarum, M. faucium e M. buccale. M. hominis e M. genitalis sono gli agenti eziologici di infezioni non specifiche del tratto urogenitale. Il Mycoplasma pneumoniae è l'agente eziologico della polmonite atipica primaria e può anche essere causa di bronchite, laringite e tracheite. Colpisce soprattutto i bambini sopra i 5-6 anni di vita e predilige, in particolare, gli adolescenti e gli adulti. Dopo un periodo di incubazione di 2-3 settimane esordisce con febbre, cefalea, scarso appetito e malessere generale. Il quadro clinico della polmonite atipica si manifesta nel 5-25% delle persone infette. L'agente è ubiquitario, molto contagioso e è trasmesso per via aerea.

Specie	Malattia	Sintomi (p.es.)	Via di trasmissione
Mycoplasma pneumoniae	Infezioni del tratto respiratorio per Mycoplasma pneumoniae	Febbre, mal di testa, e una tosse persistente. Infezioni del tratto respiratorio: Da infezione asintomatica da faringiti, bronchiti, groppa, tracheobronchite, polmonite e polmonite atipica primaria	Trasmissione: aerea; Trasmesso da gocce infette nell'aria

L'infezione o la presenza di un agente patogeno può essere identificata da:
- Microscopia:
- Sierologia: p.es.ELISA

2. USO PREVISTO

Il Mycoplasma pneumoniae IgG ELISA è un kit per la determinazione qualitativa degli anticorpi specifici della classe IgG per Mycoplasma pneumoniae nel siero o plasma (citrato, eparina) umano.

3. PRINCIPIO DEL TEST

La determinazione immunoenzimatico qualitativa degli anticorpi specifici si basa sulla tecnica ELISA (d'inglese Enzyme-linked immunosorbent assay).
Piastre di Microtitolazione sono rivestiti con antigeni specifici che si legano agli anticorpi presenti nel campione. Dopo aver lavato i pozzetti per rimuovere tutto il materiale campione non legato, il coniugato di perossidasi di rafano (HRP) è aggiunto. Questo coniugato si lega agli anticorpi catturati. In una seconda fase di lavaggio coniugato, non legato è rimosso. Il complesso immunitario formato dal coniugato legato sarà evidenziato aggiungendo tetrametilbenzidina (TMB) substrato che dà una colorazione blu.
L'intensità di questa colorazione è direttamente proporzionale alla quantità di anticorpi specifici presenti nel campione. Acido solforico è aggiunto per bloccare la reazione. Questo produce un cambiamento di colore dal blu al giallo. Assorbanza a 450/620 nm è letto utilizzando un fotometro di Piastre di Microtitolazione ELISA.

4. MATERIALI

4.1. Reagenti forniti

- **Piastre di Microtitolazione:** 12 strisce divisibili in 8 pozzetti, con adesi antigeni della Mycoplasma pneumoniae; dentro una busta d'alluminio richiudibile.
- **Tampone di Diluizione del Campione IgG:** 1 flacone contenente 100 mL di tampone fosfato (10 mM) per diluire i campioni; pH 7,2 ± 0,2; colore giallo; pronto all'uso; tappo bianco; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Soluzione Bloccante:** 1 flacone contenente 15 mL di acido solforico, 0,2 mol/L, pronto all'uso; tappo rosso.
- **Tampone di Lavaggio (20x conc.):** 1 flacone contenente 50 mL di un tampone fosfato concentrato 20 volte (0,2 M) per il lavaggio dei pozzetti; pH 7,2 ± 0,2; tappo bianco.
- **Coniugato:** 1 flacone contenente 20 mL di anticorpi IgG anti-umani, coniugati a perossidasi in tampone fosfato (10 mM); colore azzurro; pronto all'uso; tappo nero.
- **Soluzione Substrato TMB:** 1 flacone contenente 15 mL di 3,3`,5,5`-Tetrametilbenzidina (TMB), < 0,1 %; pronto all'uso; tappo giallo.
- **Controllo Positivo:** 1 flacone da 2 mL controllo; colore giallo; tappo rosso; pronto all'uso; ≤ 0,02% (v/v) MIT.
- **Controllo Cut-off:** 1 flacone da 3 mL controllo; colore giallo; tappo verde; pronto all'uso; ≤ 0,02% (v/v) MIT.
- **Controllo Negativo:** 1 flacone da 2 mL controllo; colore giallo; tappo blu; pronto all'uso; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Le indicazioni di pericolo e consigli di prudenza vedi capitolo 12.1.

Per le sostanze potenziali pericolose si prega di leggere la scheda di dati di sicurezza.

4.2. Accessori forniti

- 1 pellicola adesiva
- 1 istruzione per l'uso
- 1 schema della piastra

4.3. Materiali e attrezzature necessari

- Fotometro per Piastre di Microtitolazione con filtri da 450/620 nm
- Incubatrice 37 °C
- Lavatore, manuale o automatico, di Piastre di Microtitolazione
- Micropipette per l'uso tra 10-1000 µL
- Vortex-Mixer
- Acqua distillata
- Provette monouso

5. MODALITÀ DI CONSERVAZIONE

Conservare il kit a 2...8 °C. I reagenti aperti sono stabili fino alla data di scadenza indicata sull'etichetta quando sono conservati a 2...8 °C.

6. PREPARAZIONE DEI REAGENTI

È molto importante, portare tutti i reagenti e campioni a temperatura ambiente (20…25 °C) e mescolare prima di iniziare il test.

6.1. Piastre di Microtitolazione

Le strisce divisibili sono rivestite con gli antigeni della Mycoplasma pneumoniae. Immediatamente dopo la rimozione degli strisce necessari, le strisce rimanenti devono essere sigillare nuovamente in un foglio di alluminio insieme con il sacchetto di gel di silice conservati a 2...8 °C.

6.2. Tampone di Lavaggio (20x conc.)

Diluire il Tampone di Lavaggio 1+19; per esempio: 10 mL del Tampone di Lavaggio + 190 mL di acqua distillata. Il Tampone di Lavaggio diluito è stabile per 5 giorni a temperatura ambiente (20…25 °C). Se cristalli appaiono nel concentrato, riscaldare la soluzione a 37 °C per esempio in un bagnomaria. Mescolare bene prima della diluizione.

6.3. Soluzione Substrato TMB

La soluzione sta pronta all'uso e deve essere conservata a 2...8 °C, al riparo dalla luce. La soluzione deve essere incolore o potrebbe avere un leggero colore blu chiaro. Se il substrato diventa blu, potrebbe essere stato contaminato e non può essere utilizzato nel test.

7. PRELIEVO E PREPARAZIONE DEI CAMPIONI

Per questo test si prega di usare campioni di siero o plasma (citrato, eparina) umano. Se il test è fatto entro 5 giorni dal prelievo i campioni possono essere conservati tra 2...8 °C. Altrimenti devono essere aliquotati e congelati tra (-70…-20 °C). Se i campioni sono conservati congelati, mescolare bene i campioni scongelati prima del test. Evitare cicli ripetuti di congelamento/scongelamento.
L'inattivazione dei campioni per mezzo del calore non è raccomandata.

7.1. Diluizione dei campioni

Prima del test, diluire i campioni 1+100 con Tampone di Diluizione del Campione IgG. Per esempio, pipettare nelle provette 10 µL di campione + 1 mL di Tampone di Diluizione del Campione IgG e mescolare bene (Vortex).

8. PROCEDIMENTO

Leggere bene le istruzioni per l'uso **prima** di iniziare il teste. L'affidabilità dei risultati dipende dalla stretta aderenza le istruzioni per l'uso di prova come descritto. La seguente procedura è stata validata per l'esecuzione manuale. Per un'esecuzione su strumentazione automatica si consiglia di incrementare il numero di lavaggi de 3 a 5 volte e il volume del Tampone di Lavaggio da 300 a 350 µL per evitare effetti di lavaggio. Prestare attenzione al capitolo 12. Stabilire innanzitutto il piano di distribuzione e identificazione dei campioni e standards/controlli (è raccomandato determinare in duplicato) sullo schema della piastra fornito con il kit. Inserire i pozzetti necessari nel supporto.

Eseguire il test nell'ordine stabilito dalle istruzioni, senza ritardi.

Sul pipettaggio utilizzare puntali nuovi e puliti per ogni campione e standard/controllo.

Regolare l'incubatore a 37 ± 1 °C.

1. Pipettare 100 µL di standard/controllo e di campione diluito nei relativi pozzetti. Usare il pozzetto A1 per il Bianco-substrato.
2. Coprire i pozzetti con la pellicola adesiva, fornita nel kit.
3. **Incubare 1 ora ± 5 min a 37 ± 1°C.**
4. Al termine dell'incubazione, togliere la pellicola e aspirare il liquido dai pozzetti. Successivamente lavare i pozzetti tre volte con 300 µL di Tampone di Lavaggio. Evitare che la soluzione trabocchi dai pozzetti. L'intervallo tra il lavaggio e l'aspirazione deve essere > 5 sec. Dopo il lavaggio picchiettare delicatamente i pozzetti su una carta assorbente per togliere completamente il liquido, prima del passo successivo.

   Attenzione: Il lavaggio è una fase molto importante. Da lavaggio insufficiente risulta una bassa precisione e risultati falsi!
5. Pipettare 100 µL di Coniugato in tutti i pozzetti, escludendo quello con il Bianco-substrato (Blank) A1.
6. **Incubare per 30 min a temperatura ambiente (20...25 °C).** Non esporre a fonti di luce diretta.
7. Ripetere il lavaggio secondo punto 4.
8. Pipettare 100 µL di Soluzione Substrato TMB in tutti i pozzetti.
9. **Incubare precisamente per 15 min a temperatura ambiente (20...25 °C) al buio.** Un colore blu verifica a causa della reazione enzimatica.
10. Pipettare 100 µL di Soluzione Bloccante in tutti i pozzetti, nello stesso ordine della Soluzione Substrato TMB, in tal modo un cambiamento di colore dal blu al giallo si verifica.
11. Misurare l'assorbanza a 450/620 nm entro 30 min dopo l'aggiunta della Soluzione Bloccante.

8.1. Misurazione

Regolare il fotometro per le Piastre di Microtitolazione ELISA **a zero** usando il substrato-Bianco (Blank).

Se, per motivi tecnici, non è possibile regolare il fotometro per le Piastre di Microtitolazione a zero usando il Bianco-substrato, il valore de assorbanza de questo deve essere sottratto dai valori dell'assorbanza da tutti i valori delle altre assorbanze per ottenere risultati affidabili!

Misurare l'assorbanza di tutti i pozzetti a **450 nm** e inserire tutti i valori misurati nello schema della piastra.

È raccomandato fare le misurazioni delle onde **bichrome** (due colori). Utilizzando la lunghezza d'onda de 620 nm come misura di riferimento.

Dove sono state misurate in doppio, calcolare **la media delle assorbanze**.

9. RISULTATI

9.1. Validazione del test

Affinché un test possa essere considerato valido, le presenti Istruzioni per l'uso devono essere rigorosamente seguite e devono essere soddisfatti i seguenti criteri:

- **Substrato Bianco (Blank)**: Valore di assorbanza **< 0,100**
- **Controllo Negativo**: Valore di assorbanza **< 0,200 e < Cut-off**
- **Controllo Cut-off**: Valore di assorbanza **0,150 – 1,300**
- **Controllo Positivo**: Valore di assorbanza **> Cut-off**

Se non sono soddisfatti questi criteri, il test non è valido e deve essere ripetuto.

9.2. Calcolo dei risultati

Il Cut-off è la media dei valori di assorbanza dei Controlli Cut-off.

Esempio: Valore di assorbanza del Controllo Cut-off 0,44 + valore di assorbanza del Controllo Cut-off 0,42 = 0,86/2 = 0,43
Cut-off = 0,43

9.2.1. Risultati in unità [NTU]

$$\frac{\text{Assorbanza media del campione} \times 10}{\text{Cut-off}} = \text{[unità NovaTec = NTU]}$$

Esempio: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretazione dei risultati

Cut-off	10 NTU	-
Positivo	> 11 NTU	Anticorpi contro il patogeno sono presenti. C'è stato un contatto con l'antigene (patogeno resp. vaccino).
Zona grigia	9 – 11 NTU	Anticorpi contro il patogeno non è stato possibile rilevare chiaramente. Si consiglia di ripetere il test con un nuovo campione in 2-4 settimane. Se il risultato è nuovamente nella zona grigia, il campione viene giudicato come **negativo**.
Negativo	< 9 NTU	Il campione non contiene anticorpi contro il patogeno. Un precedente contatto con l'antigene (patogeno resp. vaccino) è improbabile.
La diagnosi di una malattia infettiva non deve essere fatta soltanto sulla risultanza di un unico test. È importante considerare anche l'anamnesi ed i sintomi del paziente. I risultati del test da pazienti immunosoppressi e neonati hanno un valore limitato.		

9.3.1. Isotipi degli anticorpi e Stato dell'infezione

Sierologia	Significato
IgM	Caratteristica della risposta primaria dell'anticorpo Alto titolo IgM con basso titolo IgG: →suggerisce una infezione molto recente o acuta Raro: → IgM persistente
IgG	Caratteristica della risposta secondaria dell'anticorpo Può persistere per diversi anni Alto titolo IgG con basso titolo IgM: → può indicare un'infezione passata
IgA	Sono prodotte a livello delle mucose in tutto il corpo (⇒ barriera protettiva) Solitamente sono prodotte all'inizio dell'infezione

10. CARATTERISTICHE DEL TEST

I risultati si riferiscono al gruppo di campioni investigato; questi non sono specifiche garantite.

Per ulteriori informazioni su caratteristiche del test, si prega, di contattare NovaTec Immundiagnostica GmbH.

10.1. Precisione

Intradosaggio	**n**	**Media (OD)**	**CV (%)**
#1	24	0,434	3,80
#2	24	1,240	4,08
#3	24	1,635	6,03
Interdosaggio	**n**	**Media (NTU)**	**CV (%)**
#1	12	32,58	5,73
#2	12	31,56	13,32
#3	12	4,36	11,10

10.2. Specificità diagnostica

La specificità diagnostica è la probabilità del test di fornire un risultato negativo in assenza di analita specifici.
La specificità diagnostica è 97,8% (95% intervallo di confidenza: 92,29% - 99,73%).

10.3. Sensibilità diagnostica

La sensibilità diagnostica è la probabilità del test di fornire un risultato positivo alla presenza di analita specifici.
La sensibilità diagnostica è 100% (95% intervallo di confidenza: 96,87% - 100%).

10.4. Possibili interferenze

Campioni emolitici, lipidici et itterici contenenti fino a 10 mg/mL di emoglobina, 5 mg/mL di trigliceridi e 0,5 mg/mL di bilirubina non hanno presentato fenomeni d'interferenza nel presente test.

10.5. Reattività crociata

L'investigazione di un gruppo di campioni con attività di anticorpi contro parametri potenzialmente interferenti non ha rivelato alcuna evidenza di risultati falsamente positivi dovuto a reattività crociata.

11. LIMITAZIONI

Una contaminazione da microorganismi o ripetuti cicli di congelamento-scongelamento possono alterare i valori delle assorbanze.

12. PRECAUZIONI E AVVERTENZE

- La procedura analitica, le informazioni, le precauzioni e le avvertenze contenute nelle istruzioni per l'uso devono essere seguite scrupolosamente. L'uso dei kit con analizzatori e attrezzature similari deve essere previamente convalidato. Qualunque cambiamento nello scopo, nel progetto, nella composizione o struttura e nella procedura analitica, così come qualunque uso dei kit in associazione ad altri prodotti non approvati dal produttore non è autorizzato; l'utilizzatore stesso è responsabile di questi eventuali cambiamenti. Il produttore non è responsabile per falsi risultati e incidenti che possano essere causati da queste ragioni. Il produttore non è responsabile per qualunque risultato ottenuto attraverso esame visivo dei campioni dei pazienti.
- Solo per uso diagnostico in-vitro.
- Tutti i materiali di origine umana o animale devono essere considerati potenzialmente contagiosi e infettivi.
- Tutti gli elementi di origine umana sono stati trovati non reattivi con Anti-HIV-Ab, Anti-HCV-Ab e HBsAg.
- Non scambiare reagenti e Piastre di Microtitolazione di lotti diversi.
- Non utilizzare reagenti d'altri produttori insieme con i reagenti di questo kit.
- Non usare dopo la data di scadenza.
- Utilizzare soltanto punte per pipette, distributori, e articoli da laboratorio puliti.
- Non scambiare i tappi dei flaconi, per evitare contaminazione crociata.
- Richiudere i flaconi immediatamente dopo l'uso per evitare la vaporizzazione e contaminazione.
- Una volta aperti e dopo relativo stoccaggio verificare i reagenti per una loro eventuale contaminazione prima dell'uso.
- Per evitare contaminazioni crociate e risultati erroneamente alti pipettare i campioni e reagenti con molta precisione nei pozzetti senza spruzzi.
- L'ELISA è progettato solo per il personale qualificato che segue le norme di buona pratica di laboratorio (Good Laboratory Practice, GLP).
- Per un ulteriore controllo di qualità interno ogni laboratorio dovrebbe inoltre utilizzare campioni noti.

12.1. Nota di sicurezza per i reagenti contenenti sostanze pericolose

I reagenti possono contenere CMIT/MIT (3:1) o MIT (vedi capitolo 4.1)
Pertanto, si applicano le seguenti indicazioni di pericolo e le consigli di prudenza.

Attenzione	H317	Può provocare una reazione allergica cutanea.
	P261	Evitare di respirare gli aerosol.
	P280	Indossare guanti/ indumenti protettivi.
	P302+P352	IN CASO DI CONTATTO CON LA PELLE: lavare abbondantemente con sapone acqua.
	P333+P313	In caso di irritazione o eruzione della pelle: consultare un medico.
	P362+P364	Togliere tutti gli indumenti contaminati e lavarli prima di indossarli nuovamente.

Ulteriori informazioni sono disponibili nella scheda di dati di sicurezza.

12.2. Smaltimento

In genere tutte le sostanze chimiche sono considerati rifiuti pericolosi. Lo smaltimento è regolato da leggi nazionali. Per ulteriori informazioni contattare l'autorità locale.

13. INFORMAZIONI PER GLI ORDINI

Numero del prodotto: MYCG0350 Mycoplasma pneumoniae IgG ELISA (96 determinazioni)

ESPAÑOL

1. INTRODUCCIÓN

Las Mycoplasma son procariotas con la caracteristica principal de no tener pared celular. Por esta facultad se forma la nueva clase de los “Mollicutes” (mollis: suave, cutis: piel). La familia de los Mycoplasmataceae se divide en los géneros Mycoplasma y Ureaplasma. En los Mycoplasma se conocen más de 80 especies dentro de las más importantes como patógenos para el hombre son M. pneumoniae, M. hominis y M. genitalis. Aparte de estos patógenos obligatorios existen varias especies que son facultativo patógeno (M. orale, M. salivarium, M. faucium, M. buccale).

M. pneumoniae produce infecciones del tracto respiratoiro (neumonías atípicas), M. hominis y M. genitalis infectan al tracto urogenital. M. pneumoniae coloniza la traquea y los bronquios destruyendo el epitelio sin entrar en el tejido peribronquial. Las infiltraciones en el tejido peribronquial son probablemente reacciones fuertes del sistema inmunitario con presencia de linfocitos, células plasma y macrófagos. Muchas veces se muestran fenómenos autoinmunitarios en forma de la formación de anticuerpos con reacciones cruzadas con tejidos corporales. Después de un período de incubación medio de 3 semanas se producen en el 75% de los casos infecciones gripales fuertes con faringitis o traqueobronquitis. Del 5 al 25 % de los casos solamente de desarolla una neumonía atípica que comienza con cansancio, dolores de cabeza, fiebre y tos pertinante.

El M. pneumoniae no pertenece a la flora normal del hombre. Las Mycoplasma son de manifestación mundial y se transmiten con alta contagiosidad por el aire y por gotats de fluido contaminado. El grupo más afectado son los jovenes de 5 a 20 años. Las infecciones dentro de una familia o en comunidades se explican por la alta contagiosidad y el modo de infección. Son comunes las reinfecciones.

Especies	Enfermedad	Síntomas (p.ej.)	Vía de transmisión
M. pneumoniae	Enfermedad respiratoria por Mycoplasma pneumoniae	Fiebre, dolor de cabeza y tos persistente Enfermedad respiratoria, de infección asintomática a resfriados, faringitis, bronquitis, krupp, traqueobronquitis, pneumonitis y neumonía atípica primaria	A erógena, por gotitas de fluido contaminado

La infección o la presencia de un patógeno puede identificarse mediante:

- Microscopia
- Serología: p.ej. ELISA

2. USO PREVISTO

El enzimoinmunoensayo Mycoplasma pneumoniae IgG ELISA se utiliza para la determinación cualitativa de anticuerpos IgG específicos contra Mycoplasma pneumoniae en suero o plasma (citrato, heparina) humano.

3. PRINCIPIO DEL ENSAYO

La determinación inmunoenzimática cualitativa de anticuerpos específicos se basa en la técnica ELISA (Enzyme-linked Immunosorbent Assay).
Las Placas de Microtitulación están recubiertas con antígenos específicos unen a los anticuerpos de la muestra. Después de lavar los pocillos para eliminar todo el material de muestra no unida, el conjugado de peroxidasa de rábano (HRP) se añade. Este conjugado se une a los anticuerpos capturados. En una segunda etapa de lavado se retira el conjugado no unido. El complejo inmune formado por el conjugado unido se visualiza añadiendo substrato tetrametilbencidina (TMB), que da un producto de reacción azul.
La intensidad de este producto es proporcional a la cantidad de anticuerpos específicos en la muestra. se añade ácido sulfúrico para detener la reacción. Esto produce un cambio de color de azul a amarillo. La extinción a 450/620 nm se mide con un fotómetro de Placa de Microtitulación ELISA.

4. MATERIALES

4.1. Reactivos suministrados

- **Placa de Microtitulación :** 12 tiras de 8 pocillos rompibles, recubiertos con antígenos de Mycoplasma pneumoniae, en bolsa de aluminio.
- **Tampón de Dilución de Muestras IgG:** 1 botella de 100 mL de solución de tampón de fosfato (10 mM) para diluir la muestra; pH 7,2 ± 0,2; color amarillo; listo para ser utilizado; tapa blanca; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solución de Parada:** 1 botella de 15 mL de ácido sulfúrico, 0,2 mol/L, listo para ser utilizado; tapa roja.
- **Tampón de Lavado (20x conc.):** 1 botella de 50 mL de una solución de tampón de fosfato 20x concentrado (0,2 M) para lavar los pocillos; pH 7,2 ± 0,2; tapa blanca.
- **Conjugado:** 1 botella de 20 mL de conjugado de anticuerpos IgG anti-humano con peroxidasa en tampón de fosfato (10 mM); color azul; tapa negra; listo para ser utilizado.
- **Solución de Sustrato de TMB:** 1 botella de 15 mL 3,3',5,5'-tetrametilbenzindina (TMB), < 0,1 %; listo para ser utilizado; tapa amarilla.
- **Control Positivo:** 1 botella de 2 mL control; color amarillo; tapa roja; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Cut-off:** 1 botella de 3 mL control; color amarillo; tapa verde; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Negativo:** 1 botella de 2 mL control; color amarillo; tapa azul; listo para ser utilizado; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Para indicaciones de peligro y consejos de prudencia consulte el cap. 12.1.
Para sustancias potencialmente peligrosas por favor revise la ficha de datos de seguridad.

4.2. Accesorios suministrados

- 1 lámina autoadhesiva
- 1 instrucciones de uso
- 1 esquema de la placa

4.3. Materiales e instrumentos necesarios

- Fotómetro de Placa de Microtitulación con filtros de 450/620 nm
- Incubadora 37 °C
- Dispositivo de lavado manual o automático de Placas de Microtitulación
- Micropipetas para uso de (10-1000 µL)
- Mezcladora Vortex
- Agua destilada
- Tubos de plástico desechables

5. ESTABILIDAD Y ALMACENAJE

Almacene el kit a 2...8 °C. Los reactivos abiertos son estables hasta la fecha de caducidad indicada en la etiqueta cuando se almacena a 2...8 °C.

6. PREPARACIÓN DE LOS REACTIVOS

Es muy importante llevar todos los reactivos y las muestras a temperatura ambiente (20...25 °C) y mezclarlos antes de ser utilizados!

6.1. Placa de Microtitulación

Las tiras rompibles están recubiertas con antígeno de Mycoplasma pneumoniae. Inmediatamente después de la eliminación de las tiras, las tiras restantes deben sellarse de nuevo en el papel de aluminio junto con la bolsita di dióxio de silicio y almacenar a 2...8 °C.

6.2. Tampón de Lavado (20x conc.)

Diluir el Tampón de Lavado 1+19; por ejemplo 10 mL del Tampón de Lavado + 190 mL de agua destilada. El Tampón de Lavado diluido es estable durante 5 días a temperatura ambiente (20…25 °C). En caso de aparecer cristales en el concentrado, calentar la solución a 37 °C, por ejemplo, en un baño María. Mezclar bien antes de la dilución.

6.3. Solución de Sustrato de TMB

La solución está lista para su uso y debe almacenarse a 2...8 °C, protegida de la luz. La solución debe ser incolora o podría tener un color ligeramente azul claro. Si el sustrato se convierte en azul, es posible que haya sido contaminado y no puede ser utilizado en el ensayo.

7. TOMA Y PREPARACIÓN DE LAS MUESTRAS

Usar muestras de suero o plasma (citrato, heparina) humano. Si el ensayo se realiza dentro de 5 días después de la toma de sangre, las muestras pueden ser almacenadas a 2...8 °C, en caso contrario deben ser alicuotadas y almacenadas congeladas (-70...-20 °C). Agitar bien las muestras descongeladas antes de diluirlas. Evitar congelaciones y descongelaciones repetidas.
No se recomienda la inactivación por calor de las muestras.

7.1. Dilución de las muestras

Antes del ensayo, las muestras tienen que estar diluidas en relación 1 + 100 con el Tampón de Dilución de Muestras IgG, p. e. 10 µL de la muestra con 1 mL de Tampón de Dilución de Muestras IgG, mezclar bien con la mezcladora Vortex.

8. PROCEDIMIENTO

Por favor, leer cuidadosamente las instrucciones de uso del ensayo **antes** de realizarlo. Para el buen funcionamiento de la técnica es necesario seguir las instrucciones. El siguiente procedimiento es válido solamente para el método manual. Si se realiza el ensayo en los sistemas automáticos de ELISA es aconsejable elevar el número de lavados de tres hasta cinco veces y el volumen de Tampón de Lavado de 300 µL a 350 µL para excluir efectos de lavado. Preste atención al capítulo 12. Antes de comenzar, especificar exactamente la repartición y posición de las muestras y de los estándares/controles (se recomienda determinar en duplicado) en el esquema de la placa suministrada. Usar la cantidad necesaria de tiras o pocillos e insertarlos en el soporte.

Realizar el ensayo en el orden indicado y sin retraso.

Para cada paso de pipeteado en los estándares/controles y en las muestras, usar siempre puntas de pipeta de un solo uso.

Graduar la incubadora a 37 ± 1°C.

1. Pipetear 100 µL de estándares/controles y muestras en los pocillos respectivos. Dejar el pocillo A1 para el blanco.
2. Recubrir las tiras con los autoadhesivos suministrados.
3. **Incubar 1 h ± 5 min a 37 ± 1°C.**
4. Después de la incubación, retirar el autoadhesivo, aspirar el líquido de la tira y lavarla tres veces con 300 µL del Tampón de Lavado. Evitar el rebosamiento de los pocillos. El intervalo entre lavado y aspiración debe ser > 5 segundos. Para sacar el líquido restante de las tiras, es conveniente sacudirlas sobre papel absorbente.

   Nota: El lavado es muy importante! Un mal lavado insuficiente provoca una baja precisión y resultados falsamente elevados!
5. Pipetar 100 µL de conjugado en cada pocillo con excepción del blanco substrato A1.
6. **Incubar 30 min a la temperatura ambiente (20…25 °C).** Evitar la luz solar directa.
7. Repetir el lavado como en el paso numero 4.
8. Pipetar 100 µL de la Solución de Sustrato de TMB en todos los pocillos.
9. **Incubar exactamente 15 min en oscuridad a temperatura ambiente (20...25 °C).** Un color azul se produce en las muestras positivas debido a la reacción enzimática.
10. Pipetear en todos los pocillos 100 µL de la Solución de Parada en el mismo orden y mismo intervalo de tiempo como con el Solución de Sustrato de TMB, por lo tanto un cambio de color de azul a amarillo se produce.
11. Medir la extinción con 450/620 nm en un periodo de 30 min después de añadir la Solución de Parada.

8.1. Medición

Ajustar el fotómetro de Placa de Microtitulación ELISA al cero utilizando el Blanco.

Si por razones técnicas el fotómetro de Placa de Microtitulación de ELISA no se puede ajustar a cero utilizando el Blanco, el valor de la absorbancia de este debe ser sustraído de los demás valores de absorbancia medidos con el fin de obtener resultados fiables!

Medir la **extinción** de todos los pocillos con **450 nm** y anotar los resultados de los estándares/controles y de las muestras en el esquema de la placa.

Es aconsejable realizar la medición **bicromática** a una longitud de onda de referencia de 620 nm.

Si se efectuaron análisis en duplicado o múltiples, hay que calcular **el promedio de los valores de extinción** de los pocillos correspondientes.

9. CÁLCULO DE LOS RESULTADOS

9.1. Criterios de validez del ensayo

Para que un ensayo se considere válido, deben seguirse estrictamente las presentes instrucciones de uso y deben cumplirse los siguientes criterios:

- **Blanco:** valor de la extinción **< 0,100**
- **Control Negativo:** valor de la extinción **< 0,200 y < Cut-off**
- **Control Cut-off:** valor de la extinción **0,150 – 1,300**
- **Control Positivo:** valor de la extinción **> Cut-off**

Si estos criterios no se cumplen, la prueba no es váida y deberá repetirse.

9.2. Cálculo del valor de la medición

El Cut-off se obtiene de los valores de la extinción de lo Control Cut-off.

Ejemplo: 0,42 OD Control Cut-off + 0,44 OD Control Cut-off = 0,86:2 = 0,43

Cut-off = 0,43

9.2.1. Resultados en unidades [NTU]

$$\frac{\text{Promedio valor de la extinción de la muestra} \times 10}{\text{Cut-off}} = \text{[NovaTec-unidades = NTU]}$$

Ejemplo: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretación de los resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Los anticuerpos contra el patógeno están presentes. Ha producido un contacto con el antígeno (patógeno resp. vacuna).
Zona intermedia	9 – 11 NTU	Los anticuerpos contra el patógeno no se pudieron detectar claramente. Se recomienda repetir la prueba con una muestra fresca en 2 a 4 semanas. Si el resultado es de nuevo en la zona intermedia, la muestra se considera como **negativa**.
Negativo	< 9 NTU	La muestra no contiene anticuerpos contra el patógeno. Un contacto previo con el antígeno (patógeno resp. vacuna) es poco probable.
El diagnóstico de una infección no solamente se debe basar en el resultado del ensayo. Es necesario considerar la anamnésis y la sintomatología del paciente junto al resultado serológico. Estos resultados sólo tienen valor restringido en pacientes inmunodeprimidos o en neonatos.		

9.3.1. Isotipos de anticuerpo y Estado de la Infección

Serología	Significado
IgM	Característica de la respuesta primaria del anticuerpo Alto título de IgM con bajo título de IgG → sugieren una infección muy reciente o aguda Raras: → persistente IgM
IgG	Característica de la respuesta secundaria del anticuerpo Pueden persistir por varios años El alto título de IgG con bajo título de IgM: → pueden indicar una infección pasada
IgA	Producida en el revestimiento mucoso en todo el cuerpo (⇒ Barrera Protectora) Usualmente producida tempranamente en el transcurso de la infección

10. CARACTERÍSTICAS DEL ENSAYO

Los resultados están basados en el grupo de pruebas investigado; no se trata de especificaciones garantizadas.

Para obtener más información sobre las características del ensayo, por favor, entre en contacto NovaTec Immundiagnostica GmbH.

10.1. Precisión

Intra ensayo	**n**	**Promedio (E)**	**CV (%)**
#1	24	0,434	3,80
#2	24	1,240	4,08
#3	24	1,635	6,03
Inter ensayo	**n**	**Promedio (NTU)**	**CV (%)**
#1	12	32,58	5,73
#2	12	31,56	13,32
#3	12	4,36	11,10

10.2. Especificad diagnóstica

La especificidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado negativo en ausencia del analítico específico. Es 97,8% (95% Intervalo de confianza: 92,29% - 99,73%).

10.3. Sensibilidad de diagnóstico

La sensibilidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado positivo en presencia del analítico específico. Es 100% (95% Intervalo de confianza: 96,87% - 100%).

10.4. Interferencias

Las muestras lipémicas, ictéricas e hemolíticas no mostraron interferencias con este equipo ELISA hasta una concentración de 5 mg/mL para triglicéridos, de 0,5 mg/mL para bilirrubina y de 10 mg/mL hemoglobina.

10.5. Reactividad cruzada

Pruebas realizadas con un panel de muestras con distinta actividad de anticuerpos para estudiar parámetros de reactividad no dieron falsos positivos debidos a reactividad cruzada.

11. LIMITACIONES DEL ENSAYO

Una contaminación de las muestras con bacterias, o una congelación y descongelación repetida pueden producir cambios en los valores de la extinción.

12. PRECAUCIONES Y ADVERTENCIAS

- El procedimiento, la información, las precauciones y los avisos de las instrucciones de uso han de ser seguidas estrictamente. La utilización de equipos con analizadores y equipamiento similar tiene que ser validada. No se autorizan cambios en el diseño, composición y procedimiento, así como cualquier utilización en combinación con otros productos no aprobados por el fabricante; el usuario debe hacerse responsable de estos cambios. El fabricante no responderá ante falsos resultados e incidentes debidos a estas razones. El fabricante no responderá ante cualquier resultado por análisis visual de las muestras de los pacientes.
- Solo para diagnostico in vitro.
- Todos los materiales de origen humano o animal deberán ser considerados y tratados como potencialmente infecciosos.
- Todos los componentes de origen humano han sido examinados y resultaron no reactivos a anticuerpos contra el VIH, VHC y HbsAG.
- No intercambiar reactivos y Placa de Microtitulación de cargas diferentes.
- No usar reactivos de otro fabricante para este ensayo.
- No usar después de la fecha de caducidad.
- Sólo usar recambios de pipetas, dispensadores y materiales de laboratorio limpios.
- No intercambiar las tapas de los diferentes reactivos, para evitar la contaminación cruzada.
- Para evitar la evaporación y una contaminación microbiana, cierre inmediatamente las botellas después de usarlas.
- Después de abrirlas y posterior almacenaje, asegurarse de que no existe contaminación microbiana antes de seguir usándolas.
- Para evitar contaminaciones cruzadas y resultados erróneamente aumentados, Pipetear cuidadosamente las muestras y los reactivos en los pocillos sin salpicar.
- El ELISA sólo está diseñado para personal cualificado siguiendo las normas de buenas prácticas de laboratorio (Good Laboratory Practice,GLP).
- Para un mayor control de calidad interno, cada laboratorio deberá utilizar además muestras conocidas.

12.1. Nota de seguridad para los reactivos que contienen sustancias peligrosas

Los reactivos pueden contener CMIT/MIT (3:1) o MIT (consulte el cap. 4.1)
Por lo tanto, se aplican las indicaciones de peligro y consejos de prudencia.

Atención

H317	Puede provocar una reacción alérgica en la piel.
P261	Evitar respirar el aerosol.
P280	Llevar guantes/ prendas de protección
P302+P352	EN CASO DE CONTACTO CON LA PIEL: Lavar con abundante jabón agua.
P333+P313	En caso de irritación o erupción cutánea: Consultar a un médico.
P362+P364	Quitar las prendas contaminadas y lavarlas antes de volver a usarlas.

Se puede encontrar más información en la ficha de datos de seguridad.

12.2. Indicaciones para la eliminación de residuos

Por regla general, los productos químicos y las preparaciones son residuos peligrosos. Su eliminación esta sometida a las leyes y los decretos nacionales sobre la eliminación de residuos. Las autoridades informan sobre la eliminación de residuos peligroso.

13. INFORMACIONES PARA PEDIDOS

N° del producto:	MYCG0350	Mycoplasma pneumoniae IgG ELISA (96 determinaciones)

PORTUGUÊS

1. INTRODUÇÃO

Os micoplasmas pertencem à classe Mollicutes composta por três famílias e quatro géneros distintos, um dos quais é o Mycoplasma com mais de 60 espécies. Os Micoplasmas são os menores organismos de vida livre conhecidos (300 a 500 nm de diâmetro) e, ao contrário das bactérias normais não possuem uma parede celular. Os Micoplasmas são parasitas extracelulares, especialmente nas membranas mucosas, que podem causar infecções em humanos, animais, plantas e culturas celulares. O Mycoplasma pneumoniae é essencialmente um agente patogénico respiratório (obrigatório) em humanos envolvendo a nasofaringe, garganta, traqueia, brônquios, bronquíolos e alvéolos. Outros Micoplasmas, M. buccale, M. faucium, M. orale e M. salivarium são comensais na cavidade oral. O Mycoplasma hominis e o Ureaplasma urealyticum habitam principalmente o trato genital e podem actuar como invasores oportunistas. O M. pneumoniae é de longe o agente patogénico mais importante deste grupo. A infecção por M. pneumoniae ocorre em todo o mundo, a sua epidemiologia tem sido estudada principalmente no E.U.A., Europa e Japão. As infecções são endémicas nas grandes áreas urbanas, e são observados aumentos da epidemia em intervalos variáveis. Foi estimado que o M. pneumoniae seja a causa de 15-20% de todas as pneumonias, a taxa é mais elevada em crianças e adultos jovens. 74% das infecções por M. pneumoniae são assintomáticas, a reinfecção pode ocorrer. A imunidade adquirida naturalmente à infecção por M. pneumoniae parece ser de duração limitada (2-3 anos).

Espécies	Doença	Sintomas (p.ex.)	Via de transmissão
M. pneumoniae	Doença respiratória Mycoplasma pneumonia	Febre, cefaléia e tosse persistente Doença respiratória: de infecção assintomática ao resfriados, faringite, bronquite, crup, traqueobronquite, pneumonite e pneumonia primária atípica	Por via aérea, a transmissão do vírus ocorre por infecção por gotículas;

Infecção ou presença de patógeno pode ser identificada por:

- Microscopia
- Serologia: p.ex.ELISA

2. UTILIZAÇÃO PRETENDIDA

O kit Mycoplasma pneumoniae IgG ELISA destina-se à determinação qualitativa de anticorpos da classe IgG contra Mycoplasma pneumoniae no soro ou plasma (citrato, heparina) humanos.

3. PRINCÍPIO DO ENSAIO

A determinação imunoenzimática qualitativa de anticorpos específicos é baseado na técnica de ELISA (do inglês Enzyme-linked Immunosorbent Assay).
As Placas de Microtitulação são revestidas com antigénios específicos que se ligam os anticorpos correspondentes da amostra. Após lavagem dos poços, para remover todo o material de amostra não ligado, um conjugado de peroxidase de rábano (HRP) é adicionado. Este conjugado se liga aos anticorpos capturados. Num segundo passo de lavagem o conjugado não ligado é removido. O complexo imune formado pelo conjugado ligado é visualizado por adição de substrato de tetrametilbenzidina (TMB), o que dá um produto de reacção azul.
A intensidade deste produto é proporcional à quantidade de anticorpos específicos da amostra. O ácido sulfúrico é adicionado para parar a reacção. Isso produz uma mudança de cor de azul para amarelo.
Absorvância a 450/620 nm é lida utilizando um fotômetro de Placa de Microtitulação ELISA.

4. MATERIAIS

4.1. Reagentes fornecidos

- **Placa de Microtitulação:** 12 tiras de 8 poços, destacáveis e quebráveis, revestidas com antigénio de Mycoplasma pneumoniae, em bolsas de folha de alumínio com fecho.
- **Tampão de Diluição de Amostra IgG:** 1 frasco contendo 100 mL de tampão fosfato (10 mM) para diluição da amostra, pH 7,2 ± 0,2; de cor amarela; pronto a usar; tampa branca; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solução de Bloqueio:** 1 frasco contendo 15 mL ácido sulfúrico; 0,2 mol/L; pronto a usar; tampa vermelha.
- **Tampão de Lavagem (conc. 20x):** 1 frasco contendo 50 mL de um tampão fosfato (0,2 M); concentrado 20 vezes (pH 7,2 ± 0,2) para a lavagem dos poços; tampa branca.
- **Conjugado:** 1 frasco contendo 20 mL de anticorpo IgG anti-humana marcados com peroxidase no tampão fosfato (10 mM); de cor azul, pronto a usar; tampa preta.
- **Solução Substrato TMB:** 1 frasco contendo 15 mL de 3,3',5,5'-tetrametilbenzidina (TMB), < 0,1 %; pronto a usar; tampa amarela.
- **Controle Positivo:** 1 frasco contendo 2 mL controle; de cor amarela; pronto a usar; tampa vermelha; ≤ 0,02% (v/v) MIT.
- **Controle Cut-off:** 1 frasco contendo 3 mL controle; de cor amarela; pronto a usar; tampa verde; ≤ 0,02% (v/v) MIT.
- **Controle Negativo:** 1 frasco contendo 2 mL controle; de cor amarela; pronto a usar; tampa azul; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Para advertências de perigo e recomendaçaões de prudência ver capitulo 12.1.
Para substâncias potencialmente perigosas verifique a ficha de dados de segurança.

4.2. Materiais fornecidos

- 1 Película de cobertura
- 1 Instruções de uso
- 1 Layout da placa

4.3. Materiais e Equipamento necessários

- Fotômetro de Placa de Microtitulação ELISA, equipado para a medição da absorvância a 450/620 nm
- Incubadora 37 °C
- Equipamento manual ou automático para a lavagem das Placa de Microtitulação
- Pipetas para dispensar volumes entre 10 e 1000 µL
- Agitador de tubos tipo Vortex
- Água destilada
- Tubos descartáveis

5. ESTABILIDADE E ARMAZENAMENTO

Armazene o kit a 2...8 °C. Os reagentes abertos são estáveis até o prazo de validade impresso no rótulo quando armazenado a 2...8 °C.

6. PREPARAÇÃO DOS REAGENTES

É muito importante deixar todos os reagentes e amostras estabilizar à temperatura ambiente (20...25 °C) misturá-los antes de iniciar o teste!

6.1. Placa de Microtitulação

As tiras quebraveis são revestidas com antigénio Mycoplasma pneumoniae. Imediatamente após a remoção das tiras necessarias, as tiras restantes devem ser lacradas de novo na folha de alumínio juntamente com o saquinho de silicio fornecido e armazenadar a 2...8 °C.

6.2. Tampão de Lavagem (conc. 20x)

Diluir o Tampão de Lavagem 1+19; por exemplo. 10 mL do Tampão de Lavagem + 190 mL de água destilada. O Tampão de Lavagem diluído é estavel durante 5 dias à temperatura ambiente (20…25 °C). Caso apareça cristais no concentrado, aquecer a solução a 37 °C por exemplo, em banho Maria. Misture bem antes da diluição.

6.3. Solução Substrato TMB

A solução está pronta para uso e tem de ser armazenada à 2...8 °C, protegida da luz. A solução deve ser incolor ou poderia ter uma ligeira coloração azul claro. Se o substrato se transforma em azul, pode ter sido contaminado e não pode ser usado no teste.

7. COLHEITA E PREPARAÇÃO DAS AMOSTRAS

Usar com este ensaio amostras de soro ou plasma (citrato, heparina) humanos. Se o ensaio for realizado dentro de 5 dias após colheita da amostra, o espécime deve ser mantido a 2…8 °C; caso contrário devem ser alicotadas e armazenadas congeladas (-70...-20 °C). Se as amostras forem armazenadas congeladas, misturar bem as amostras descongeladas antes de testar. Evitar congelar e descongelar repetidamente.
Não é recomendada a inactivação por calor das amostras.

7.1. Diluição das amostras

Antes de testar todas as amostras devem ser diluídas 1 + 100 com Tampão de Diluição de Amostra IgG. Dispensar 10 µL de amostra e 1 mL de Tampão de Diluição de Amostra IgG em tubos para obter uma diluição 1 + 100 e misturar meticulosamente com um vortex.

8. PROCEDIMENTO DO ENSAIO

Por favor, ler atentamente as instruções de uso **antes** de realizar o teste. A fiabilidade dos resultados depende da adesão estrita ao as instruções de uso, conforme descritas. O procedimento de ensaio a seguir está validado apenas para o procedimento manual. Se o teste for realizado em sistemas automáticos para teste ELISA é recomendável aumentar os passos de lavagem de três até cinco e o volume do Tampão de Lavagem de 300 µL para 350 µL para evitar efeitos de lavagem. Preste atenção ao capítulo 12. Antes de iniciar o teste, o plano de distribuição e identificação de todas as amostras e calibradores/controles (é recomendado determinar em duplicidade) deve ser cuidadosamente estabelecido no Layout da placa fornecida no kit. Seleccionar o número necessário de tiras ou poços e inserir os mesmos no suporte.

Realizar todas as etapas do teste na ordem indicada e sem atrasos significativos.

Na pipetagem deve ser utilizada uma ponta limpa e descartável para dispensar cada controle e amostra.

Ajustar a incubadora para 37 ± 1 °C.

1. Dispensar 100 µL dos calibradores/controles e das amostras diluídas nos poços respectivos. Deixar o poço A1 vazio para o branco substrato.
2. Cobrir os poços com a película fornecida no kit.
3. **Incubar durante 1 hora ± 5 min a 37 ± 1 °C.**
4. Quando terminar a incubação, remover a película, aspirar o conteúdo dos poços e lavar cada poço três vezes com 300 µL de Tampão de Lavagem. Evitar que os poços de reacção transbordem. O intervalo entre a lavagem e a aspiração deve ser > 5 seg. No final, retirar cuidadosamente o fluido restante batendo delicadamente as tiras sobre papel absorvente, antes da próxima etapa!

   Nota: A lavagem é muito importante! Lavagem insuficiente resulta em baixa precisão e falsos resultados.
5. Dispensar 100 µL de Conjugado em todos os poços, excepto no poço do Branco substrato A1.
6. **Incubar durante 30 min à temperatura ambiente (20...25°C).** Não expor diretamente à luz solar.
7. Repetir a etapa 4.
8. Dispensar 100 µL de Solução Substrato TMB em todos os poços.
9. **Incubar durante exactamente 15 min à temperatura ambiente (20...25°C) e no escuro.** A cor azul devido a uma reacção enzimática.
10. Dispensar 100 µL de Solução de Bloqueio em todos os poços, pela mesma ordem e com a mesma velocidade a que foi dispensada a Solução Substrato TMB,desse modo uma mudança de cor de azul para amarelo ocorre.
11. Medir a absorvância a 450/620 nm dentro de 30 min após a adição da Solução de Bloqueio.

8.1. Medição

Ajustar o fotômetro para Placa de Microtitulação ELISA **a zero** usando o **Branco substrato .**

Se - devido à razões técnicas – o fotômetro para Placa de Microtitulação ELISA não puder ser ajustado a zero usando o Branco substrato, valor da absorvância deste deve ser subtraido de todos os outros valores de absorvância medidos de forma a obter resultados fiáveis!

Medir a absorvância de todos os poços a **450 nm** e registar os valores da absorvância para cada calibrador/controle e amostra no Layout da placa.

É recomendado fazer a medição **dicromática** usando como referência um comprimento de onda de 620 nm.

Se determinações duplas foram realizadas, calcular **os valores médios de absorvância**.

9. RESULTADOS

9.1. Critérios de validação do ensaio

Para que um ensaio seja considerado válido, estas Instruções de Uso devem ser rigorosamente seguidas, e os seguintes critérios devem ser cumpridos:

- **Branco substrato**: Valor de Absorvância **< 0,100**
- **Controle Negativo**: Valor de Absorvância **< 0,200 e < Cut-off**
- **Controle Cut-off**: Valor de Absorvância **0,150 – 1,300**
- **Controle Positivo**: Valor de Absorvância **> Cut-off**

Se estes critérios não forem cumpridos, o teste não é válido e deve ser repetido.

9.2. Cálculo dos Resultados

O Cut-off é o valor médio da absorvância das determinações do Controle Cut-off.

Exemplo: Valor da absorvância do Controle Cut-off 0,42 + valor da absorvância do Controle Cut-off 0,44 = 0,86: 2 = 0,43
Cut-off = 0,43

9.2.1. Resultados em Unidades [NTU]

$$\frac{\text{Valor da absorvância (média) da amostra} \times 10}{\text{Cut-off}} = \text{[Unidades NovaTec = NTU]}$$

Exemplo: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretação dos Resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Os anticorpos contra o agente patogênico estão presente. Houve um contacto com o antigénio (patógeno resp vacina).
Zona cinzenta	9 – 11 NTU	Os anticorpos contra o agente patogênico não puderam ser claramente detectados. Recomenda-se a repetir o teste com uma amostra fresca em 2 a 4 semanas. Se o resultado estiver novamente dentro da zona cinzenta, a amostra é julgada como **negativa**.
Negativo	< 9 NTU	A amostra não contém os anticorpos contra o agente patogênico. Um contato prévio com o antígeno (patógeno resp. vacina) é improvável.
O diagnóstico de uma doença infecciosa não deve ser estabelecido com base num único resultado do teste. Um diagnóstico preciso deve ter em consideração a história clínica, a sintomatologia bem como dados serológicos. Em pacientes imunossuprimidos e recém-nascidos os dados serológicos têm apenas valor restrito.		

9.3.1. Isotipos de anticorpos e Estado da Infecção

Sorologia	Significado
IgM	Característica da resposta primária do anticorpo Alto título de IgM com baixo título de IgG: → sugere uma infecção muito recente ou aguda Raros: → persistente IgM
IgG	Característica da resposta secundária do anticorpo Podem persistir por vários anos Alto título de IgG com baixo título de IgM: → pode indicar uma infecção passada
IgA	Eles são produzidos a nível das mucosas em todo o corpo (⇒ barreira protectora) Geralmente são producidas no inicio infecção

10. CARACTERÍSTICAS DE DESEMPENHO ESPECÍFICAS

Os resultados referem-se aos grupos de amostras investigados; estas não são especificações garantidas.

Para mais informações sobre as características de desempenho específicas, por favor, entre em contato NovaTec Immundiagnostica GmbH.

10.1. Precisão

Intra ensaio	**n**	**Média (E)**	**CV (%)**
#1	24	0,434	3,80
#2	24	1,240	4,08
#3	24	1,635	6,03
Inter ensaio	**n**	**Média (NTU)**	**CV (%)**
#1	12	32,58	5,73
#2	12	31,56	13,32
#3	12	4,36	11,10

10.2. Especificidade Diagnóstica

A especificidade diagnóstica é definida como a probabilidade do ensaio ser negativo na ausência do analito específico.
É de 97,8% (95% Intervalo de confiança: 92,29% - 99,73%).

10.3. Sensibilidade Diagnóstica

A sensibilidade diagnóstica é definida como a probabilidade do ensaio ser positivo na presença do analito específico.
É de 100% (95% Intervalo de confiança: 96,87% - 100%).

10.4. Interferências

Não são observadas interferências com amostras hemolisadas, lipémicas ou ictéricas até uma concentração de hemoglobina de 10 mg/mL, de triglicerídeos de 5 mg/mL e de bilirrubina de 0,5 mg/mL.

10.5. Reacção cruzada

A investigação do painel de amostras com atividades de anticorpos em parâmetros com potencial de reação cruzada não revelou nenhuma evidencia de resultados falso-positivos devido a reações cruzadas.

11. LIMITAÇÕES DO PROCEDIMENTO

Contaminação bacteriana ou a repetição de ciclos de congelação-descongelação do espécime podem afectar os valores da absorvância.

12. PRECAUÇÕES E AVISOS

- O procedimento do teste, as informações, as precauções e avisos nas instruções para utilização têm de ser rigorosamente seguidas. O uso de kits de teste com analisadores e equipamento similar tem de ser validado. Qualquer alteração no desenho, composição e procedimento do teste bem como qualquer utilização em combinação com outros produtos não aprovados pelo fabricante não estão autorizados; o próprio utilizador é responsável por tais alterações. O fabricante não é legalmente responsável por resultados falsos e incidentes originados por estes motivos. O fabricante não é legalmente responsável por quaisquer resultados obtidos por análise visual das amostras dos pacientes.
- Apenas para uso no diagnóstico in-vitro.
- Todos os materiais de origem humana ou animal devem ser considerados e tratados como potencialmente infectantes.
- Todos os componentes de origem humana usados para a produção destes reagentes foram testados para anticorpos anti-HIV, anticorpos anti-HCV e HBsAg e foram considerados não-reactivos.
- Não trocar e/ou juntar reagentes ou Placa de Microtitulação de lotes de produção diferentes.
- Nenhuns reagentes de outros fabricantes devem ser usados juntamente com reagentes deste kit de teste.
- Não usar reagentes após a data de validade indicada no rótulo.
- Usar apenas pontas de pipeta, dispensadores e material de laboratório limpos.
- Não trocar as tampas dos frascos dos reagentes para evitar contaminação cruzada.
- Fechar firmemente os frascos dos reagentes imediatamente após a utilização para evitar evaporação e contaminação microbiana.
- Após a primeira abertura e armazenamento subsequente verificar se existe contaminação microbiana dos frascos do conjugado e dos calibradores/controles antes de utiliza-los novamente.
- Para evitar contaminação-cruzada e resultados falsamente elevados, pipetar as amostras dos pacientes e dispensar o reagentes precisamente nos poços sem salpicar.
- O ELISA é projetado apenas para pessoal qualificado seguindo os padrões de boas práticas de laboratório (Good Laboratory Practice, GLP).
- Para um controle de qualidade interno adicional cada laboratório deve utilizar amostras conhecidas.

12.1. Nota de segurança para reagentes que contenham substâncias perigosas

Os reagentes podem conter CMIT/MIT (3:1) ou MIT (ver capitulo 4.1)
Portanto, as seguintes advertências de perigo e recomendações de prudência aplicam-se.

Atenção

H317	Pode provocar uma reacção alérgica cutânea.
P261	Evitar respirar os aerossóis.
P280	Usar luvas de protecção/ vestuário de protecção.
P302+P352	SE ENTRAR EM CONTACTO COM A PELE: lavar abundantemente com sabão água.
P333+P313	Em caso de irritação ou erupção cutânea: consulte um médico.
P362+P364	Retirar a roupa contaminada e lavá-la antes de a voltar a usar.

Mais informações podem ser encontradas na ficha de dados de segurança.

12.2. Considerações de Eliminação

Resíduos de químicos e preparações são geralmente considerados como resíduos perigosos. A eliminação deste tipo de resíduos está regulada por leis e normativas nacionais e regionais. Contactar as autoridades locais ou empresas de gestão de resíduos as quais podem aconselhar sobre como eliminar resíduos perigosos.

13. INFORMAÇÃO DE PEDIDO

Prod. No.: MYCG0350 Mycoplasma pneumoniae IgG ELISA (96 Determinações)

BIBLIOGRAPHY / LITERATUR / BIBLIOGRAPHIE / BIBLIOGRAFIA / BIBLIOGRAFÍA / BIBLIOGRAFIA

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ABBREVIATIONS / ABKÜRZUNGEN / ABRÉVIATIONS / ABBREVIAZIONI / ABREVIACIÓNES / ABREVIATURAS

CMIT	5-chloro-2-methyl-4-isothiazolin-3-one
MIT	2-methyl-2H-isothiazol-3-one

SYMBOLS KEY / SYMBOLSCHLÜSSEL / EXPLICATION DES SYMBOLES / LEGENDA / SIMBOLOS / TABELA DE SIMBOLOS

	Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por
IVD	In Vitro Diagnostic Medical Device / In Vitro Diagnosticum / Dispositif médical de diagnostic in vitro / Diagnostico in vitro / Producto para diagnóstico In vitro / Dispositivo Médico para Diagnóstico In Vitro
LOT	Lot Number / Chargenbezeichnung / Numéro de lot / Lotto / Número de lote / Número de lote
	Expiration Date / Verfallsdatum / Date de péremption / Scadenza / Fecha de caducidad / Data de Validade
	Storage Temperature / Lagertemperatur / Température de conservation / Temperatura di conservazione / Temperatura de almacenamiento / Temperatura de Armazenamento
CE	CE Mark / CE-Zeichen / Marquage CE / Marchio CE / Marca CE / Marca CE
REF	Catalogue Number / Katalog Nummer / Référence du catalogue / Numero di codice / Número de Catálogo / Número de Catálogo
	Consult Instructions for Use / Arbeitsanleitung beachten / Consulter la notice d'utilisation / Consultare le istruzioni per l'uso / Consulte las Instrucciones de Uso / Consultar as Instruções de Utilização
MTP	Microtiterplate / Mikrotiterplatte / Plaque de Microtitrage / Piastre di Microtitolazione / Placa de Microtitulación / Placa de Microtitulación
CONJ	Conjugate / Konjugat / Conjugué / Coniugato / Conjugado / Conjugado
CONTROL | -	Negative Control / Negativkontrolle / Contrôle Négatif / Controllo Negativo / Control Negativo / Controle Negativo
CONTROL | +	Positive Control / Positivkontrolle / Contrôle Positif / Controllo Positivo / Control Positivo / Controle Positivo
CUT OFF	Cut-off Control / Cut-off Kontrolle / Contrôle Cut-off / Controllo Cut-off / Control Cut-off / Controle Cut-off
DIL | G	IgG Sample Dilution Buffer / IgG-Probenverdünnungspuffer / Tampon de Dilution d'Échantillon IgG / Tampone di Diluizione del Campione IgG / Tampón de Dilución de Muestras IgG/ Tampão de Diluição de Amostra IgG
SOLN | STOP	Stop Solution / Stopplösung / Solution d'Arrêt / Soluzione Bloccante / Solución de Parada/ Solução de Bloqueio
SUB | TMB	TMB Substrate Solution / TMB-Substratlösung / Solution de Substrat TMB / Soluzione Substrato TMB / Solución de Sustrato de TMB / Solução Substrato TMB
WASH | BUF | 20x	Washing Buffer 20x concentrated / Waschpuffer 20x konzentriert / Tampon de Lavage concentré 20 x / Tampone di Lavaggio concentrazione x20 / Tampón de Lavado concentrado x20 / Tampão de Lavagem concentrada 20x
	Contains sufficient for "n" tests / Ausreichend für "n" Tests / Contenu suffisant pour "n" tests / Contenuto sufficiente per "n" saggi / Contenido suficiente para "n" tests / Conteúdo suficiente para "n" testes

SUMMARY OF TEST PROCEDURE / KURZANLEITUNG TESTDURCHFÜHRUNG / RÉSUMÉ DE LA PROCEDURE DE TEST / SCHEMA DELLA PROCEDURA / RESUMEN DE LA TÉCNICA / RESUMO DO PROCEDIMENTO DE TESTE

SCHEME OF THE ASSAY

Mycoplasma pneumoniae IgG ELISA

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and standards/controls on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Negative Control	Cut-off Control	Positive Control	Sample (diluted 1+100)
Negative Control	-	100 µL	-	-	-
Cut-off Control	-	-	100 µL	-	-
Positive Control	-	-	-	100 µL	-
Sample (diluted 1+100)	-	-	-	-	100 µL
Cover wells with foil supplied in the kit **Incubate for 1 h at 37 ± 1 °C** Wash each well three times with 300 µL of Washing Buffer					
Conjugate	-	100 µL	100 µL	100 µL	100 µL
Incubate for 30 min at room temperature (20...25 °C) Do not expose to direct sunlight Wash each well three times with 300 µL of Washing Buffer					
TMB Substrate Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Incubate for exactly 15 min at room temperature (20...25 °C) in the dark					
Stop Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

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NovaTec Immundiagnostica GmbH

NovaLisa®

Mycoplasma pneumoniae IgM

ELISA

CE

Only for in-vitro diagnostic use

Product Number: MYCM0350 (96 Determinations)

ENGLISH

1. INTRODUCTION

The mycoplasms belong to the class Mollicutes comprising three distinct families and four genera, one of which is Mycoplasma with over 60 species. Mycoplasmae are the smallest freeliving organisms known (300 to 500 nm in diameter) and unlike regular bacteria they lack a cell wall. Mycoplasms are extracellular parasites, especially on mucous membranes, which can cause infections in hu-mans, animals, plants, and cell cultures. Mycoplasma pneumoniae is primarily a respiratory pathogen (obligat) in humans involving the nasopharynx, throat, trachea, bronchi, bronchioles, and alveoli. Other Mycoplasmae, M. buccale, M. faucium, M. orale and M. salivarium are commensals in the oral cavity. Mycoplasma hominis and Ureaplasma urealyticum inhabit primarily the genital tract and may act as opportunistic invaders. M. pneumoniae is by far the most important pathogen of this group. Infection with M. pneumoniae occurs worldwide, its epidemiology has been studied primarily in the USA, Europe, and Japan. Infections are endemic in larger urban areas, and epidemic increases are observed at varying intervalls. Mycoplasma pneumoniae has been estimated to cause 15-20% of all pneumoniae; the rate is highest in children and young adults. 74% of infections with M. pneumoniae are asymptomatic, reinfection may occur. Naturally acquired immunity to infection with M. pneumoniae appears to be of limited duration (2-3 years).

Species	Disease	Symptoms (e.g.)	Transmission route
M. pneumoniae	Respiratory diseases by Mycoplasma pneumoniae	Fever, headache, and a persistent cough. Respiratory tract disease: from asymptomatic infection to colds, pharyngitis, bronchitis, croup, tracheobronchitis, pneumonitis and primary atypical pneumonia	Transmitted by aerosol droplets

Infection or presence of pathogen may be identified by:

- Microscopy
- Serology: e.g. ELISA

2. INTENDED USE

The Mycoplasma pneumoniae IgM ELISA is intended for the qualitative determination of IgM class antibodies against Mycoplasma pneumoniae in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Mycoplasma pneumoniae antigens; in resealable aluminium foil.
- **IgM Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidase labelled antibody to human IgM in phosphate buffer (10 mM); coloured red; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplates
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Mycoplasma pneumoniae antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgM Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgM Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43
Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU (Units)

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

9.3.1. Antibody Isotypes and State of Infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection
IgA	Produced in mucosal linings throughout the body (⇒ protective barrier) Usually produced early in the course of the infection

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	**n**	**Mean (E)**	**CV (%)**
#1	24	0.526	5.02
#2	24	0.905	6.20
#3	24	1.074	6.34
Interassay	**n**	**Mean (NTU)**	**CV (%)**
#1	12	19.56	7.08
#2	12	18.16	13.13
#3	12	5.28	7.31

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.

It is 99.29% (95% confidence interval: 96.11% - 99.98%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.

It is 100% (95% confidence interval: 95.01% - 100%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning		
	H317	May cause an allergic skin reaction.
	P261	Avoid breathing spray
	P280	Wear protective gloves/ protective clothing.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: MYCM0350 Mycoplasma pneumoniae IgM ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Die Mykoplasmen gehören zur Klasse der Mollicutes, welche drei unterschiedliche Familien und vier Genera einschließt. Eines davon ist Mycoplasma mit über 60 Arten. Mycoplasmen sind die kleinsten bekannten frei lebenden Organismen (300 bis 500 nm Durchmesser). Die besitzen im Gegensatz zu Bakterien keine Zellwand. Mycoplasmen sind extrazelluläre Parasiten speziell der Schleimhäute und können bei Menschen, Tieren, Pflanzen und in Zellkulturen Infektionen hervorrufen. Mycoplasma pneumoniae ist in erster Linie ein obligat pathogener Erreger respiratorischer Erkrankungen unter Einbeziehung von Nasopharynx, Rachen, Luftröhre, Bronchien, Bronchiolen und Aveolen. Andere Mycoplasmen (M. buccale, M. faucium, M. orale, M. salivarium) sind Kommensalen in der Mundhöhle. Mycoplasma hominis und Ureaplasma urealyticum besiedeln vorwiegend den Genitaltrakt und können als opportunistische Invasoren auftreten. Mycoplasma pneumoniae ist mit Abstand das wichtigste Pathogen dieser Gruppe. Infektionen mit M. pneumoniae treten weltweit auf, deren Epidemiologie ist hauptsächlich in den USA, Europa und Japan untersucht worden. In größeren urbanen Regionen sind die Infektionen endemisch und epidemieartige Zuwächse werden in unregelmäßigen Abständen beobachtet. Es wird geschätzt, dass M. pneumoniae 15 bis 20 % aller Pneumonien verursacht; die Rate ist bei Kindern und jungen Erwachsenen am größten. 74 % der Infektionen mit M. pneumoniae verlaufen symptomlos; Reinfektionen können auftreten. Eine natürlich erworbene Immunität gegen Infektionen mit M. pneumoniae hält nur für 2 bis 3 Jahre an.

Spezies	Erkrankung	Symptome (z.B.)	Infektionsweg
M. pneumoniae	Erkrankungen der Atemwege duch Mycoplasma pneumoniae	Fieber, Kopfschmerzen und hartnäckiger Husten. Atemwegserkrankung, von asymptomatischer Infektion über Erkältungskrankheit, Pharyngitis, Bronchitis, Krupp, Tracheobronchitis, Pneumonitis und primäre atypische Pneumonie	Aerogen durch Tröpfchen

Nachweis des Erregers bzw. der Infektion durch:

- Mikroskopie
- Serologie: z.B. ELISA

2. VERWENDUNGSZWECK

Der Mycoplasma pneumoniae IgM ELISA ist für den qualitativen Nachweis spezifischer IgM-Antikörper gegen Mycoplasma pneumoniae in humanem Serum oder Plasma (Citrat, Heparin) bestimmt.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.

4. MATERIALIEN

4.1. Mitgelieferte Reagenzien

- **Mikrotiterplatte:** 12 teilbare 8er-Streifen, beschichtet mit Mycoplasma pneumoniae Antigenen; in wieder verschließbarem Aluminiumbeutel.
- **IgM-Probenverdünnungspuffer:** 1 Flasche mit 100 mL Phosphatpuffer (10 mM) zur Probenverdünnung; pH 7,2 ± 0,2; anti-human IgG (RF- Absorbens); grün gefärbt; gebrauchsfertig; weiße Verschlusskappe; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Stopplösung:** 1 Flasche mit 15 mL Schwefelsäure, 0,2 mol/L; gebrauchsfertig; rote Verschlusskappe.
- **Waschpuffer (20x konz.):** 1 Flasche mit 50 mL eines 20-fach konzentrierten Phosphatpuffers (0,2 M), zum Waschen der Kavitäten; pH 7,2 ± 0,2; weiße Verschlusskappe.
- **Konjugat:** 1 Flasche mit 20 mL Peroxidase-konjugierten Antikörpern gegen humanes IgM in Phosphatpuffer (10 mM); rot gefärbt; gebrauchsfertig; schwarze Verschlusskappe.
- **TMB-Substratlösung:** 1 Flasche mit 15 mL 3,3`,5,5`-Tetramethylbenzidin (TMB), < 0,1 %; gebrauchsfertig; gelbe Verschlusskappe.
- **Positivkontrolle:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; rote Verschlusskappe; gebrauchsfertig; ≤ 0,02% (v/v) MIT.
- **Cut-off Kontrolle:** 1 Fläschchen mit 3 mL Kontrolle; gelb gefärbt; grüne Verschlusskappe; gebrauchsfertig; ≤ 0,02% (v/v) MIT.
- **Negativkontrolle:** 1 Fläschchen mit 2 mL Kontrolle; gelb gefärbt; blaue Verschlusskappe; gebrauchsfertig; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Für Gefahren- und Sicherheitshinweise siehe 12.1.
Für potenzielle Gefahrstoffe überprüfen Sie bitte das Sicherheitsdatenblatt.

4.2. Mitgeliefertes Zubehör

- 1 selbstklebende Abdeckfolie
- 1 Arbeitsanleitung
- 1 Plattenlayout

4.3. Erforderliche Materialien und Geräte

- Mikrotiterplatten-Photometer mit Filtern 450/620 nm
- Inkubator 37 °C
- Manuelle oder automatische Waschvorrichtung für Mikrotiterplatten
- Mikropipetten (10 - 1000 µL)
- Vortex-Mischer
- Destilliertes Wasser
- Plastikröhrchen für den einmaligen Gebrauch

5. STABILITÄT UND LAGERUNG

Testkit bei 2...8 °C lagern. Die geöffneten Reagenzien sind bis zu den auf den Etiketten angegebenen Verfallsdaten verwendbar, wenn sie bei 2...8 °C gelagert werden.

6. VORBEREITUNG DER REAGENZIEN

Es ist sehr wichtig, alle Reagenzien und Proben vor ihrer Verwendung auf Raumtemperatur (20...25 °C) zu bringen und zu mischen!

6.1. Mikrotiterplatte

Die abbrechbaren Streifen sind mit Mycoplasma pneumoniae Antigenen beschichtet. Nicht verbrauchte Vertiefungen im Aluminiumbeutel zusammen mit dem Trockenmittel sofort wieder verschließen und bei 2...8 °C lagern.

6.2. Waschpuffer (20x konz.)

Der Waschpuffer ist im Verhältnis 1 + 19 zu verdünnen; z.B. 10 mL Waschpuffer + 190 mL destilliertes Wasser.
Der verdünnte Puffer ist bei Raumtemperatur (20…25 °C) 5 Tage haltbar. Sollten Kristalle im Konzentrat auftreten, die Lösung z.B. in einem Wasserbad auf 37 °C erwärmen und vor dem Verdünnen gut mischen.

6.3. TMB-Substratlösung

Die gebrauchsfertige Lösung ist bei 2...8 °C vor Licht geschützt aufzubewahren. Die Lösung ist farblos, kann aber auch leicht hellblau sein. Sollte die TMB-Substratlösung blau sein, ist sie kontaminiert und kann nicht im Test verwendet werden.

7. ENTNAHME UND VORBEREITUNG DER PROBEN

Es sollten humane Serum- oder Plasmaproben (Citrat, Heparin) verwendet werden. Werden die Bestimmungen innerhalb von 5 Tagen nach Blutentnahme durchgeführt, können die Proben bei 2...8 °C aufbewahrt werden, sonst aliquotieren und tiefgefrieren (-70…-20 °C). Wieder aufgetaute Proben vor dem Verdünnen gut schütteln. Wiederholtes Tiefgefrieren und Auftauen vermeiden!
Hitzeinaktivierung der Proben wird nicht empfohlen.

7.1. Probenverdünnung

Proben vor Testbeginn im Verhältnis 1 + 100 mit IgM-Probenverdünnungspuffer verdünnen, z. B. 10 µL Probe und 1 mL IgM-Probenverdünnungspuffer in die entsprechenden Röhrchen pipettieren, um eine Verdünnung von 1 + 100 zu erhalten; gut mischen (Vortex).

8. TESTDURCHFÜHRUNG

Arbeitsanleitung **vor** Durchführung des Tests sorgfältig lesen. Für die Zuverlässigkeit der Ergebnisse ist es notwendig, die Arbeitsanleitung genau zu befolgen. Die folgende Testdurchführung ist für die manuelle Methode validiert. Beim Arbeiten mit ELISA Automaten empfehlen wir, um Wascheffekte auszuschließen, die Zahl der Waschschritte von drei auf bis zu fünf und das Volumen des Waschpuffers von 300 µL auf 350 µL zu erhöhen. Kapitel 12 beachten. Vor Testbeginn auf dem mitgelieferten Plattenlayout die Verteilung bzw. Position der Proben und der Standards/Kontrollen (Doppelbestimmung empfohlen) genau festlegen. Die benötigte Anzahl von Mikrotiterstreifen (Kavitäten) in den Streifenhalter einsetzen.

Den Test in der angegebenen Reihenfolge und ohne Verzögerung durchführen.

Für jeden Pipettierschritt der Standards/Kontrollen und Proben saubere Einmalspitzen verwenden.

Den Inkubator auf 37 ± 1 °C einstellen.

1. Je 100 µL Standards/Kontrollen und vorverdünnte Proben in die entsprechenden Vertiefungen pipettieren. Vertiefung A1 ist für den Substratleerwert vorgesehen.
2. Die Streifen mit der mitgelieferten Abdeckfolie bedecken.
3. **1 h ± 5 min bei 37 ± 1 °C inkubieren.**
4. Am Ende der Inkubationszeit Abdeckfolie entfernen und die Inkubationsflüssigkeit aus den Teststreifen absaugen. Anschließend dreimal mit 300 µL Waschpuffer waschen. Überfließen von Flüssigkeit aus den Vertiefungen vermeiden. Das Intervall zwischen Waschen und Absaugen sollte > 5 sec betragen. Nach dem Waschen die Teststreifen auf Fließpapier ausklopfen, um die restliche Flüssigkeit zu entfernen.

   Beachte: Der Waschvorgang ist wichtig, da unzureichendes Waschen zu schlechter Präzision und falschen Messergebnissen führt!
5. 100 µL Konjugat in alle Vertiefungen, mit Ausnahme der für die Berechnung des Leerwertes A1 vorgesehenen, pipettieren.
6. **30 min bei Raumtemperatur (20...25 °C) inkubieren.** Nicht dem direkten Sonnenlicht aussetzen.
7. Waschvorgang gemäß Punkt 4 wiederholen.
8. 100 µL TMB-Substratlösung in alle Vertiefungen pipettieren.
9. **Genau 15 min im Dunkeln bei Raumtemperatur (20...25 °C) inkubieren.** Bei enzymatischer Reaktion findet eine Blaufärbung statt.
10. In alle Vertiefungen 100 µL Stopplösung in der gleichen Reihenfolge und mit den gleichen Zeitintervallen wie bei Zugabe der TMB-Substratlösung pipettieren, dadurch erfolgt ein Farbwechsel von blau nach gelb.
11. Die Extinktion der Lösung in jeder Vertiefung bei 450/620 nm innerhalb von 30 min nach Zugabe der Stopplösung messen.

8.1. Messung

Mit Hilfe des Substratleerwertes den **Nullabgleich** des Mikrotiterplatten-Photometers vornehmen.

Falls diese Eichung aus technischen Gründen nicht möglich ist, muss nach der Messung der Extinktionswert des Substratleerwertes von allen anderen Extinktionswerten subtrahiert werden, um einwandfreie Ergebnisse zu erzielen!

Extinktion aller Kavitäten bei **450 nm** messen und die Messwerte der Standards/Kontrollen und Proben in das Plattenlayout eintragen.

Eine **bichromatische** Messung mit der Referenzwellenlänge 620 nm wird empfohlen.

Falls Doppel- oder Mehrfachbestimmungen durchgeführt wurden, den **Mittelwert der Extinktionswerte** berechnen.

9. BERECHNUNG DER ERGEBNISSE

9.1. Testgültigkeitskriterien

Damit ein Testlauf als valide betrachtet werden kann, muss diese Gebrauchsanweisung strikt befolgt werden, und die folgenden Kriterien müssen erfüllt sein:

- **Substrat-Leerwert**: Extinktionswert **< 0,100**
- **Negativkontrolle**: Extinktionswert **< 0,200 und < Cut-off**
- **Cut-off Kontrolle**: Extinktionswert **0,150 – 1,300**
- **Positivkontrolle**: Extinktionswert **> Cut-off**

Sind diese Kriterien nicht erfüllt, ist der Testlauf ungültig und muss wiederholt werden.

9.2. Messwertberechnung

Der Cut-off ergibt sich aus dem Mittelwert der gemessenen Extinktionen der Cut-off Kontrolle.

Beispiel: 0,44 OD Cut-off Kontrolle + 0,42 OD Cut-off Kontrolle = 0,86: 2 = 0,43

Cut-off = 0,43

9.2.1. Ergebnisse in Einheiten [NTU]

$$\frac{\text{Mittlere Extinktion der Probe} \times 10}{\text{Cut-off}} = \text{[NovaTec Einheiten = NTU]}$$

Beispiel: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretation der Ergebnisse

Cut-off	10 NTU	-
Positiv	> 11 NTU	Es liegen Antikörper gegen den Erreger vor. Ein Kontakt mit dem Antigen (Erreger bzw. Impfstoff) hat stattgefunden.
Grenzwertig	9 – 11 NTU	Antikörper gegen den Erreger können nicht eindeutig nachgewiesen werden. Es wird empfohlen den Test nach 2 bis 4 Wochen mit einer frischen Patientenprobe zu wiederholen. Finden sich die Ergebnisse erneut im grenzwertigen Bereich, gilt die Probe als **negativ**.
Negativ	< 9 NTU	Es liegen keine Antikörper gegen den Erreger vor. Ein vorausgegangener Kontakt mit dem Antigen (Erreger bzw. Impfstoff) ist unwahrscheinlich.
Die Diagnose einer Infektionskrankheit darf nicht allein auf der Basis des Ergebnisses einer Bestimmung gestellt werden. Die anamnestischen Daten sowie die Symptomatologie des Patienten müssen zusätzlich zu den serologischen Ergebnissen in Betracht gezogen werden. Bei Immunsupprimierten und Neugeborenen besitzen die Ergebnisse serologischer Tests nur einen begrenzten Wert.		

9.3.1. Antikörper-Isotypen und Infektionsstatus

Serologie	Bedeutung
IgM	Typisch für Primärantwort Hoher IgM-Titer bei gleichzeitig niedrigem IgG-Titer: → Hinweis auf relativ frische Infektion Selten: → persistierendes IgM
IgG	Typisch für Sekundärantwort Können auch noch nach Jahren nachweisbar sein Hoher IgG-Titer bei gleichzeitig niedrigem IgM-Titer: → wahrscheinlich länger zurückliegende Infektion
IgA	Sezerniert in allen Schleimhäuten (⇒ Schutzbarriere) Meist früh im Verlauf einer Infektion gebildet

10. TESTMERKMALE

Die Ergebnisse beziehen sich auf die untersuchten Probenkollektive; es handelt sich nicht um garantierte Spezifikationen.

Für weitere Informationen zu den Testmerkmalen kontaktieren Sie bitte NovaTec Immundiagnostica GmbH.

10.1. Präzision

Intraassay	**n**	**Mittelwert (E)**	**Vk (%)**
#1	24	0,526	5,02
#2	24	0,905	6,20
#3	24	1,074	6,34
Interassay	**n**	**Mittelwert (NTU)**	**Vk (%)**
#1	12	19,56	7,08
#2	12	18,16	13,13
#3	12	5,28	7,31

10.2. Diagnostische Spezifität

Die diagnostische Spezifität ist definiert als die Wahrscheinlichkeit des Tests, ein negatives Ergebnis bei Fehlen des spezifischen Analyten zu liefern. Sie beträgt 99,29% ((95% Konfidenzintervall: 96,11% - 99,98%).

10.3. Diagnostische Sensitivität

Die diagnostische Sensitivität ist definiert als die Wahrscheinlichkeit des Tests, ein positives Ergebnis bei Vorhandensein des spezifischen Analyten zu liefern. Sie ist 100% (95% Konfidenzintervall: 95,01% - 100%).

10.4. Interferenzen

Hämolytische, lipämische und ikterische Proben ergaben bis zu einer Konzentration von 10 mg/mL Hämoglobin, 5 mg/mL Triglyceride und 0,5 mg/mL Bilirubin keine Interferenzen im vorliegenden ELISA.

10.5. Kreuzreaktivität

Die Untersuchung eines Probenpanels mit Antikörperaktivitäten gegen potenziell kreuzreagierende Parameter ließ keine Anzeichen von falsch-positiven Ergebnissen aufgrund von Kreuzreaktivitäten erkennen.

11. GRENZEN DES VERFAHRENS

Kontamination der Proben durch Bakterien oder wiederholtes Einfrieren und Auftauen können zu einer Veränderung der Messwerte führen.

12. SICHERHEITSMASSNAHMEN UND WARNHINWEISE

- Die Testdurchführung, die Information, die Sicherheitsmaßnahmen und Warnhinweise in der Arbeitsanleitung sind strikt zu befolgen. Bei Anwendung des Testkits auf Diagnostika-Geräten ist die Testmethode zu validieren. Jede Änderung am Aussehen, der Zusammensetzung und der Testdurchführung sowie jede Verwendung in Kombination mit anderen Produkten, die der Hersteller nicht autorisiert hat, ist nicht zulässig; der Anwender ist für solche Änderungen selbst verantwortlich. Der Hersteller haftet für falsche Ergebnisse und Vorkommnisse aus solchen Gründen nicht. Auch für falsche Ergebnisse aufgrund von visueller Auswertung wird keine Haftung übernommen.
- Nur für in-vitro-Diagnostik.
- Alle Materialien menschlichen oder tierischen Ursprungs sind als potentiell infektiös anzusehen und entsprechend zu behandeln.
- Alle verwendeten Bestandteile menschlichen Ursprungs sind auf Anti-HIV-AK, Anti-HCV-AK und HBsAg nicht-reaktiv getestet.
- Reagenzien und Mikrotiterplatten unterschiedlicher Chargen nicht untereinander austauschen.
- Keine Reagenzien anderer Hersteller zusammen mit den Reagenzien dieses Testkits verwenden.
- Nicht nach Ablauf des Verfallsdatums verwenden.
- Nur saubere Pipettenspitzen, Dispenser und Labormaterialien verwenden.
- Verschlusskappen der einzelnen Reagenzien nicht untereinander vertauschen, um Kreuzkontaminationen zu vermeiden.
- Flaschen sofort nach Gebrauch fest verschließen, um Verdunstung und mikrobielle Kontamination zu vermeiden.
- Nach dem ersten Öffnen Konjugat und Standards/Kontrollen vor weiterem Gebrauch auf mikrobielle Kontamination prüfen.
- Zur Vermeidung von Kreuzkontamination und falsch erhöhten Resultaten, Reagenzien sorgfältig in die Kavitäten pipettieren.
- Der ELISA ist nur für qualifiziertes Personal bestimmt, das den Standards der Guten Laborpraxis (GLP) folgt.
- Zur weiteren internen Qualitätskontrolle sollte jedes Labor zusätzlich bekannte Proben verwenden.

12.1. Sicherheitshinweis für Reagenzien, die Gefahrstoffe enthalten

Die Reagenzien können CMIT/MIT (3:1) oder MIT enthalten (siehe 4.1)
Daher gelten die folgenden Gefahren- und Sicherheitshinweise.

Achtung

H317	Kann allergische Hautreaktionen verursachen.
P261	Einatmen von Aerosol vermeiden.
P280	Schutzhandschuhe/ Schutzkleidung tragen.
P302+P352	BEI BERÜHRUNG MIT DER HAUT: Mit viel Seife und Wasser waschen.
P333+P313	Bei Hautreizung oder -ausschlag: Ärztlichen Rat einholen/ ärztliche Hilfe hinzuziehen.
P362+P364	Kontaminierte Kleidung ausziehen und vor erneutem Tragen waschen

Weitere Informationen können dem Sicherheitsdatenblatt entnommen werden.

12.2. Entsorgungshinweise

Chemikalien und Zubereitungen sind in der Regel Sonderabfälle. Deren Beseitigung unterliegt den nationalen abfallrechtlichen Gesetzen und Verordnungen. Die zuständige Behörde informiert über die Entsorgung von Sonderabfällen.

13. BESTELLINFORMATIONEN

Produktnummer: MYCM0350 Mycoplasma pneumoniae IgM ELISA (96 Bestimmungen)

FRANÇAIS

1. INTRODUCTION

Les mycoplasmes appartiennent à la classe des Mollicutes qui comprend trois familles distinctes et quatre genres, dont l'un est le mycoplasme avec plus de 60 espèces. Les Mycoplasmes sont les plus petits organismes libres connus (300 à 500 nm de diamètre) et contrairement aux bactéries régulières ils n'ont pas de membrane cellulaire. Les mycoplasmes sont des parasites extracellulaires, particulièrement sur les membranes muqueuses, qui peuvent causer des infections dans l'homme, les animaux, les plantes, et les cultures cellulaires. Mycoplasma pneumoniae est d'abord un pathogène respiratoire (obligatoire) dans l'homme qui peut atteindre le nasopharynx, la gorge, la trachée, les bronches, les bronchioles, et les alvéoles. D'autres mycoplasmes, M. buccale, M. faucium, M. orale et M. salivarium sont des commensales dans la cavité buccale. Mycoplasma hominis et Ureaplasma urealyticum habitent surtout la région génitale et peuvent agir en tant qu'envahisseurs opportunistes. Mycoplasma pneumoniae est de loin le microbe pathogène le plus important de ce groupe. L'infection avec Mycoplasma pneumoniae se produit dans le monde entier, son épidémiologie a surtout été étudiée aux Etats-Unis, en Europe, et au Japon. Les infections sont endémiques dans les grands secteurs urbains, et des augmentations épidémiques sont observées à intervalles variables. On a estimé que Mycoplasma pneumoniae cause 15-20% de toutes les pneumonies ; le taux est le plus élevé chez les enfants et de jeunes adultes. 74% des infections avec Mycoplasma pneumoniae sont asymptomatiques, et des réinfections peuvent se produire. L'immunité naturellement acquise contre l'infection avec Mycoplasma pneumoniae semble être de durée limitée (2 ou 3 ans).

Espèce	La maladie	Symptômes (p.ex.)	Modes de transmission
Mycoplasma pneumoniae	les maladies de région respiratoire par Mycoplasma pneumoniae	Fièvre, maux de tête et toux persistante. Maladie de région respiratoire: de l'infection asymptomatique à la pharyngite, la bronchite, le croup, la trachéo-bronchite et à la pneumonie atypique primaire	La transmission du virus se produit principalement par le contact de gouttelettes.

L'infection ou la présence d'un agent pathogène peut être identifiée par:

- Microscopie:
- Sérologie: p.ex. ELISA

2. INDICATION D'UTILISATION

La trousse Mycoplasma pneumoniae IgM ELISA est prévue pour la détection qualitative des anticorps IgM anti-Mycoplasma pneumoniae dans le sérum humain ou plasma (citrate, héparine).

3. PRINCIPE DU TEST

La détermination immunoenzymatique qualitative des anticorps spécifiques est basée sur le la technique ELISA (du anglais, Enzyme-Linked Immunosorbent Assay).
Plaques de Microtitrage sont recouvertes d'antigènes spécifiques pour lier les anticorps correspondants de l'échantillon. Après le lavage des puits pour éliminer l'échantillon détaché, le conjugué peroxydase de raifort (HRP) est ajouté. Ce conjugué se lie aux anticorps capturés. Dans une deuxième étape de lavage, le conjugué non lié est éliminé. Le complexe immun formé par le conjugué lié est visualisé par l'addition tétraméthylbenzidine (TMB) qui donne un produit de réaction bleu.
L'intensité de ce produit est proportionnelle à la quantité d'anticorps spécifiques dans l'échantillon. L'acide sulfurique est ajouté pour arrêter la réaction. Cela produit un changement du bleu au jaune. L'absorbance à 450/620 nm est lue en utilisant un photomètre de Plaque de Microtitrage ELISA.

4. MATERIEL

4.1. Réactifs fournis

- **Plaque de Microtitrage:** 12 barrettes de 8 puits sécables revêtus d'antigène de Mycoplasma pneumoniae; en sachets d'aluminium refermables.
- **Tampon de Dilution d'Échantillon IgM:** 1 flacon contenant 100 mL de tampon phosphaté (10 mM) pour la dilution de l'échantillon; pH 7,2 ± 0,2; anti-humaine IgG (RF-Absorbant); prêt à l'emploi; couleur vert; bouchon blanc; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solution d'Arrêt:** 1 flacon contenant 15 mL d'acide sulfurique, 0,2 mol/L; prêt à l'emploi; bouchon rouge.
- **Tampon de Lavage (concentré x 20):** 1 flacon contenant 50 mL d'un tampon phosphaté (0,2 M) concentré 20 fois (pH 7,2 ± 0,2) pour laver les puits; bouchon blanc.
- **Conjugué:** 1 flacon contenant 20 mL d'anticorps IgM anti-humaines conjuguées à de la peroxydase du raifort dans le tampon phosphaté (10 mM); prêt à l'emploi; couleur rouge, bouchon noir.
- **Solution de Substrat TMB:** 1 flacon contenant 15 mL de 3,3',5,5'-tétraméthylbenzidine (TMB), < 0,1 %; prêt à l'emploi; bouchon jaune.
- **Contrôle Positif:** 1 flacon contenant 2 mL contrôle; prêt à l'emploi; couleur jaune; bouchon rouge; ≤ 0,02% (v/v) MIT.
- **Contrôle Cut-off:** 1 flacon contenant 3 mL contrôle; prêt à l'emploi; couleur jaune; bouchon vert; ≤ 0,02% (v/v) MIT.
- **Contrôle Négatif:** 1 flacon contenant 2 mL contrôle; prêt à l'emploi; couleur jaune; bouchon bleu; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Pour les mentions de danger et les conseils de prudence voir chapitre 12.1.
Pour les substances potentiellement dangereuses s'il vous plaît vérifiez la fiche de données de sécurité.

4.2. Matériel fourni

- 1 couvercle autocollante
- 1 instructions d'utilisation
- 1 présentation de la plaque

4.3. Matériel et équipement requis

- Photomètre de Plaque de Microtitrage ELISA, pour mesurer l'absorbance à 450/620 nm
- Incubateur 37 °C
- Laveur manuel ou automatique pour le lavage des Plaques de Microtitrage
- Pipettes pour utilisation entre 10 et 1000 µL
- Mélangeur Vortex
- Eau distillée
- Tubes jetables

5. STABILITE ET CONSERVATION

Conserver le kit à 2...8 °C. Les réactifs ouverts sont stables jusqu'à la date de péremption indiquée sur l'étiquette lorsqu'il est conservé à 2...8°C.

6. PREPARATION DES REACTIFS

Il est très important porter tous les réactifs et échantillons à température ambiante (20 ... 25 °C) et les mélanger avant de commencer le test!

6.1. Plaque de Microtitrage

Les barrettes sécables sont revêtues d'antigène Mycoplasma pneumoniae. Immédiatement après avoir prélevé les barrettes nécessaires, les barrette restantes doivent être scellés le vide dans de feuille d'aluminium avec le sac de silicium (le déshydratant) fourni et emmagasiner à 2...8 °C.

6.2. Tampon de Lavage (conc. x 20)

Diluer le Tampon de Lavage 1+19; par exemple 10 mL du Tampon de Lavage + 190 mL d'eau distillée. Le Tampon de Lavage diluée est stable pendant 5 jours à la température ambiante (20…25 °C). Cas apparaissent des cristaux dans le concentré, chauffer la solution à 37 °C par exemple dans un bain-marie mélangez bien avant dilution.

6.3. Solution de Substrat TMB

La solution est prête à utiliser et doit être emmagasiné à 2...8 °C, à l'abri de la lumière. La solution doit être incolore ou pourrait avoir une légère couleur bleu clair. Si le substrat devient bleu, il peut avoir été contaminé et ne peut pas être utilisé dans le test.

7. PRELEVEMENT ET PREPARATION DES ECHANTILLONS

Utiliser des échantillons humains de sérum ou plasma (citrate, héparine) pour ce test. Si le test est réalisé dans les 5 jours après le prélèvement, les échantillons doivent être conservés à 2...8 °C; autrement ils doivent être aliquotés et conservés surgelés (-70…-20 °C). Si les échantillons sont conservés congelés, bien mélanger les échantillons décongelés avant le test. Éviter les cycles répétés de congélation et décongélation.
L'inactivation par la chaleur des échantillons n'est pas recommandée.

7.1. Dilution de l'échantillon

Avant du test, tous les échantillons doivent être dilués 1 + 100 avec Tampon de Dilution d'Échantillon IgM. Diluer 10 µL d'échantillon avec 1 mL I Tampon de Dilution d'Échantillon IgM dans des tubes pour obtenir une dilution 1 + 100 et mélanger soigneusement sur un Vortex.

8. PROCEDE DE TEST

Lire attentivement les instructions d'utilisation **avant de** réaliser le test. La fiabilité des résultats dépend du suivi strict d'utilisation comme décrit. La technique de test suivante a été validée uniquement pour une procédure manuelle. Si le test doit être effectué sur un systèmes automatiques pour ELISA, nous conseillons d'augmenter le nombre d'étapes de lavage de trois à cinq et le volume du Tampon de Lavage de 300 à 350 µL. Faites attention au chapitre 12. Avant de commencer le test, le plan de distribution et d'identification de tous les échantillons et les étalons/contrôles (il est recommandé déterminer en double) doivent être soigneusement établi sur la feuille présentation de la plaque prévue dans le conseil de kit. Sélectionner le nombre de barrettes ou de puits nécessaires et les placer sur le support.

Réaliser toutes les étapes du test dans l'ordre donné et sans délai.

Un embout de pipette propre et jetable doit être utilisé pour distribuer chaque étalon/contrôle et échantillon.

Régler l'incubateur à 37 ± 1 °C.

1. Pipeter 100 µL de étalons/contrôles et d'échantillons dilués dans leurs puits respectifs. Garder le puits A1 pour le blanc substrat.
2. Couvrir les puits avec le couvercle, fourni dans le kit.
3. **Incuber pendant 1 heure ± 5 minutes à 37 ± 1 °C.**
4. A la fin de l'incubation, enlever le couvercle, aspirer le contenu des puits et laver chaque puits trois fois avec 300 µL du Tampon de Lavage. Éviter les débordements des puits de réaction. L'intervalle entre le cycle de lavage et l'aspiration doit être > 5 sec. À la fin, enlever soigneusement le liquide restant en tapotant les barrettes sur du papier absorbant avant la prochaine étape.

   Note: L'étape de lavage est très importante! Un lavage insuffisant peut conduire à une précision faible et de faux résultats!
5. Pipeter 100 µL du conjugué dans tous les puits sauf le puits Blanc A1.
6. **Incuber pendant 30 minutes à température ambiante (20…25°C).** N'exposer pas à la lumière directe du soleil.
7. Répéter l'étape numéro 4.
8. Pipeter 100 µL de la Solution de Substrat TMB dans tous les puits.
9. **Incuber pendant exactement 15 minutes à température ambiante (20…25°C) dans l'obscurité.** Une couleur bleue se produit en raison d'une réaction enzymatique.
10. Pipeter 100 µL de la Solution d'Arrêt dans tous les puits dans le même ordre et à la même vitesse que pour la Solution de Substrat TMB, ainsi, il y a un changement du bleu au jaune.
11. Mesurer l'absorbance à 450/620 nm dans les 30 minutes après l'addition de la Solution d'Arrêt.

8.1. Mesure

Réglez le Photomètre de Plaque de Microtitrage ELISA **à zéro** en utilisant **le Blanc substrat.**
Si - pour des raisons techniques - le Photomètre de Plaque de Microtitrage ELISA ne peut pas être ajusté à zéro en utilisant le Blanc substrat, la valeur d'absorbance de cette doit être soustraire la valeur d'absorbance de toutes les autres valeurs d'absorbance mesurées afin d'obtenir des résultats fiables!

Mesurer l'absorbance de tous les puits **à 450 nm** et enregistrer les valeurs d'absorbance pour chaque étalon/contrôle et échantillon dans la présentation de la plaque.

Il est recommandé d'effectuer la mesure **dichromatique** utilisant 620 nm comme longueur d'onde de référence.
Si doubles déterminations ont été effectuées, calculer **les valeurs moyennes d'absorbance**.

9. RESULTATS

9.1. Critères de validation

Pour qu'une série d'analyses soit considérée comme valide, ces instructions d'utilisation doivent être strictement suivies, et les critères suivants doivent être respectés:

- **Blanc Substrat:** Valeur d'absorbance **< 0,100**
- **Contrôle Négatif**: Valeur d'absorbance **< 0,200 et < Cut-off**
- **Contrôle Cut-off**: Valeur d'absorbance **0,150 – 1,300**
- **Contrôle Positif**: Valeur d'absorbance **> Contrôle Cut-off**

Lorsque ces critères ne sont pas remplis, le test n'est pas valide et doit être recommencé.

9.2. Calcul des résultats

La valeur seuil correspond à la moyenne des valeurs d'absorbance du Contrôle Cut-off.

Exemple: 0,44 DO Contrôle Cut-off + 0,42 DO Contrôle Cut-off = 0,86: 2 = 0,43

Cut-off = 0,43

9.2.1. Résultats en unités [NTU]

$$\frac{\text{Valeur (moyenne) d'absorbance de l' échantillon} \times 10}{\text{Cut-off}} = \text{[unités NovaTec = NTU]}$$

Exemple: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interprétation des résultats

Cut-off	10 NTU	-
Positif	> 11 NTU	Les anticorps dirigés contre l'agent pathogène sont présents. Il ya eu un contact avec l'antigène (pathogène resp. vaccin).
Zone grise	9 – 11 NTU	Les anticorps dirigés contre l'agent pathogène ne pouvaient pas être détectés clairement. Il est recommandé de répéter le test avec un échantillon frais dans 2 à 4 semaines. Si le résultat est encore dans la zone grise l'échantillon est jugé **négatif**.
Negatif	< 9 NTU	L'échantillon ne contient pas d'anticorps contre l'agent pathogène. Un contact préalable avec l'antigène (pathogène resp. vaccin) est peu probable.
Le diagnostic d'une maladie infectieuse ne devrait pas être établi sur la base du résultat d'une seule analyse. Un diagnostic précis devrait prendre en considération l'histoire clinique, la symptomatologie ainsi que les données sérologiques. Les données sérologiques sont de valeur limité dans le cas des patients immunodéprimés et des nouveaux-nés.		

9.3.1. Isotypes d'anticorps et l'Etat de l'infection

Sérologie	Signification
IgM	Caractéristique de la réponse primaire du anticorps Titre élevé d'IgM avec une faible titre d'IgG: → suggère une infection très récente ou aigüe Rare: → persistante IgM
IgG	Caractéristique de la réponse secondaire du anticorps Peut persister pendant plusieurs années Des titres élevés d'IgG à faible titre d'IgM: → peuvent indiquer une infection ancienne
IgA	Ils sont produits au niveau des muqueuses dans tout le corps (⇒ barrière de protection) Habituellement ils sont produits en début d'infection

10. PERFORMANCES DU TEST

Ces résultats s'appuient sur les groupes d'échantillons étudiés; il n'agit pas de caractéristiques techniques garanties.

Pour plus d'informations sur les performances du test s'il vous plaît contactez NovaTec Immundiagnostica GmbH.

10.1. Précision

Intra-essai	**n**	**moyenne (E)**	**CV (%)**
#1	24	0,526	5,02
#2	24	0,905	6,20
#3	24	1,074	6,34
Inter-essai	**n**	**moyenne (NTU)**	**CV (%)**
#1	12	19,56	7,08
#2	12	18,16	13,13
#3	12	5,28	7,31

10.2. Spécificité diagnostique

La spécificité diagnostique est définie comme la probabilité d'obtenir un résultat négatif en l'absence d'un analyte spécifique. Elle est 99,29% (95% Intervalle de confiance: 96,11% - 99,98%).

10.3. Sensibilité diagnostique

La sensibilité diagnostique est définie comme la probabilité d'obtenir un résultat positif en présence d'un analyte spécifique. Elle est 100% (95% Intervalle de confiance: 95,01% - 100%).

10.4. Interférences

Des échantillons hémolytiques ou lipémiques ou ictériques n'ont pas montré d'interférences, avec des concentrations jusqu'à 10 mg/mL de hémoglobine, 5 mg/mL de triglycérides et 0,5 mg/mL de bilirubine.

10.5. Réaction croisée

L'étude d'un panel d'échantillons avec des anticorps dirigés contre différents paramètres interférents n'a pas révélé de preuves de résultats faussement positifs dus à des réactions croisées.

11. LIMITES DE LA TECHNIQUE

Une contamination bactérienne ou des cycles de congélation/décongélation répétés de l´échantillon peuvent affecter les valeurs d'absorption.

12. PRECAUTIONS ET AVERTISSEMENTS

- La procédure de test, l'information, les précautions et mises en garde de la notice d'emploi, doivent être suivies de façon stricte. L'utilisation de ces trousses avec des automates ou dispositifs similaires doit être validée. Aucun changement de la conception, composition et procédure de test, ainsi que l'utilisation avec d'autres produits non approuvés par le fabricant, ne sont pas autorisés; seul l'utilisateur est responsable de tels changements. Le fabricant n'est pas responsable des faux résultats et des incidents dus à ces motifs. Le fabricant n'est pas responsable des résultats fournis par analyse visuelle des échantillons des patients.
- Uniquement pour diagnostic in vitro.
- Tous les matériaux d'origine humaine ou animale doivent être considérés et traités comme étant potentiellement infectieux.
- Tous les composants d'origine humaine utilisés pour la fabrication de ces réactifs ont été analysés et ont été trouvés non réactifs en Ag HBs, en anticorps anti-VHI 1 et 2 et en anticorps anti-VHC.
- Ne pas échanger les réactifs ou les Plaque de Microtitrage provenant de différents lots de production.
- Ne pas utiliser de réactifs provenant d'autres fabricants avec les réactifs de cette trousse.
- Ne pas utiliser les réactifs après la date de péremption indiquée sur l'étiquette.
- Utiliser seulement des embouts de pipette, des distributeurs et du matériel de laboratoire propres.
- Ne pas échanger les bouchons des flacons, pour éviter la contamination croisée.
- Fermer soigneusement les flacons après utilisation pour éviter l'évaporation et la contamination microbienne.
- Avant une nouvelle utilisation, vérifier les flacons de conjugué et de étalon/contrôle, déjà utilisés, pour exclure une contamination microbienne.
- Pour éviter la contamination croisée et des résultats faussement élevés, introduire les échantillons de patients et les réactifs exactement au fond des puits sans éclabousser.
- L'ELISA est uniquement conçu pour le personnel qualifié suivant les normes de bonnes pratiques de laboratoire (Good Laboratory Practice, GLP).
- Pour un contrôle de qualité interne plus poussé, chaque laboratoire doit en outre utiliser des échantillons connus.

12.1. Note de sécurité pour les réactifs contenant des substances dangereuses

Les réactifs peuvent contenir du CMIT/MIT (3 :1) ou du MIT (voir chapitre 4.1)
Par conséquent, les mentions de danger et les conseils de prudence suivants s'appliquent.

Attenttion

H317	Peut provoquer une allergie cutanée.
P261	Éviter de respirer les aérosols.
P280	Porter des gants de protection/ des vêtements de protection.
P302+P352	EN CAS DE CONTACT AVEC LA PEAU: Laver abondamment savon à l'eau.
P333+P313	En cas d'irritation ou d'éruption cutanée: consulter un médecin.
P362+P364	Enlever les vêtements contaminés et les laver avant réutilisation.

De plus amples informations peuvent être trouvées dans la fiche de données de sécurité.

12.2. Elimination des déchets

Les résidus des produits chimiques et des préparations sont considérés en général comme des déchets dangereux. L'élimination de ce type de déchet est réglementée par des lois et réglementations nationales et régionales. Contacter les autorités compétentes ou les sociétés de gestion des déchets pour obtenir des renseignements sur l'élimination des déchets dangereux.

13. INFORMATION POUR LES COMMANDES

Référence:	MYCM0350	Mycoplasma pneumoniae IgM ELISA (96 déterminations)

ITALIANO

1. INTRODUZIONE

Il Mycoplasma pneumoniae è un membro della classe dei Mollicuti, in quanto privo di parete cellulare e rivestito soltanto da membrana plasmatica. Pertanto rappresenta la più piccola cellula vivente al momento conosciuta. La famiglia dei Mycoplasmataceae si divide nei generi Mycoplasma ed Ureaplasma. Al momento sono conosciute più di 80 specie. Le specie piú importanti e patogene per l'uomo sono: M. pneumonite, M. hominis e M. genitalis. Oltre a queste esistono altre specie eventualmente patogene: M. orale, M. salivarum, M. faucium e M. buccale. M. hominis e M. genitalis sono gli agenti eziologici di infezioni non specifiche del tratto urogenitale. Il Mycoplasma pneumoniae è l'agente eziologico della polmonite atipica primaria e può anche essere causa di bronchite, laringite e tracheite. Colpisce soprattutto i bambini sopra i 5-6 anni di vita e predilige, in particolare, gli adolescenti e gli adulti. Dopo un periodo di incubazione di 2-3 settimane esordisce con febbre, cefalea, scarso appetito e malessere generale. Il quadro clinico della polmonite atipica si manifesta nel 5-25% delle persone infette. L'agente è ubiquitario, molto contagioso e è trasmesso per via aerea.

Specie	Malattia	Sintomi (p.es.)	Via di trasmissione
Mycoplasma pneumoniae	Infezioni del tratto respiratorio per Mycoplasma pneumoniae	Febbre, mal di testa, e una tosse persistente. Infezioni del tratto respiratorio: Da infezione asintomatica da faringiti, bronchiti, groppa, tracheobronchite, polmonite e polmonite atipica primaria.	Trasmissione: aerea; Trasmesso da gocce infette nell'aria

L'infezione o la presenza di un agente patogeno può essere identificata da:

- Microscopia
- Sierologia: p.es. ELISA

2. USO PREVISTO

Il Mycoplasma pneumoniae IgM ELISA è un kit per la determinazione qualitativa degli anticorpi specifici della classe IgM per Mycoplasma pneumoniae nel siero o plasma (citrato, eparina) umano.

3. PRINCIPIO DEL TEST

La determinazione immunoenzimatico qualitativa degli anticorpi specifici si basa sulla tecnica ELISA (d'inglese Enzyme-linked immunosorbent assay).
Piastre di Microtitolazione sono rivestite con antigeni specifici che si legano agli anticorpi presenti nel campione. Dopo aver lavato i pozzetti per rimuovere tutto il materiale campione non legato, il coniugato di perossidasi di rafano (HRP) è aggiunto. Questo coniugato si lega agli anticorpi catturati. In una seconda fase di lavaggio coniugato, non legato è rimosso. Il complesso immunitario formato dal coniugato legato sarà evidenziato aggiungendo tetrametilbenzidina (TMB) substrato che dà una colorazione blu.
L'intensità di questa colorazione è direttamente proporzionale alla quantità di anticorpi specifici presenti nel campione. Acido solforico è aggiunto per bloccare la reazione. Questo produce un cambiamento di colore dal blu al giallo. Assorbanza a 450/620 nm viene letto utilizzando un fotometro di Piastre di Microtitolazione ELISA.

4. MATERIALI

4.1. Reagenti forniti

- **Piastre di Microtitolazione:** 12 strisce divisibili in 8 pozzetti, con adesi antigeni della Mycoplasma pneumoniae; dentro una busta d'alluminio richiudibile.
- **Tampone di Diluizione del Campione IgM:** 1 flacone contenente 100 mL di tampone fosfato (10 mM) per diluire i campioni; pH 7,2 ± 0,2; anti-umane IgG (mezzo di assorbimento RF); colore verde; pronto all'uso; tappo bianco; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Soluzione Bloccante:** 1 flacone contenente 15 mL di acido solforico, 0,2 mol/L, pronto all'uso; tappo rosso.
- **Tampone di Lavaggio (20x conc.):** 1 flacone contenente 50 mL di un tampone fosfato concentrato 20 volte (0,2 M) per il lavaggio dei pozzetti; pH 7,2 ± 0,2; tappo bianco.
- **Coniugato:** 1 flacone contenente 20 mL di anticorpi anti-IgM umani, coniugati a perossidasi in tampone fosfato (10 mM); colore rosso; pronto all'uso; tappo nero.
- **Soluzione Substrato TMB:** 1 flacone contenente 15 mL di 3,3`,5,5`-Tetrametilbenzidina (TMB), < 0,1 %; pronto all'uso; tappo giallo.
- **Controllo Positivo:** 1 flacone da 2 mL controllo; colore giallo; tappo rosso; pronto all'uso; ≤ 0,02% (v/v) MIT.
- **Controllo Cut-off:** 1 flacone da 3 mL controllo; colore giallo; tappo verde; pronto all'uso; ≤ 0,02% (v/v) MIT.
- **Controllo Negativo:** 1 flacone da 2 mL controllo; colore giallo; tappo blu; pronto all'uso; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Le indicazioni di pericolo e consigli di prudenza vedi capitolo 12.1.

Per le sostanze potenziali pericolose si prega di leggere la scheda di dati di sicurezza.

4.2. Accessori forniti

- 1 pellicola adesiva
- 1 istruzione per l'uso
- 1 schema della piastra

4.3. Materiali e attrezzature necessari

- Fotometro per Piastre di Microtitolazione con filtri da 450/620 nm
- Incubatrice 37 °C
- Lavatore, manuale o automatico, di Piastre di Microtitolazione
- Micropipette per l'uso tra 10-1000 µL
- Vortex-Mixer
- Acqua distillata
- Provette monouso

5. MODALITÀ DI CONSERVAZIONE

Conservare il kit a 2...8 °C. I reagenti aperti sono stabili fino alla data di scadenza indicata sull'etichetta quando sono conservati a 2...8 °C.

6. PREPARAZIONE DEI REAGENTI

È molto importante, portare tutti i reagenti e campioni a temperatura ambiente (20…25 °C) e mescolare prima di iniziare il test.

6.1. Piastre di Microtitolazione

Le strisce divisibili sono rivestite con l'antigeni della Mycoplasma pneumoniae. Immediatamente dopo la rimozione degli strisce necessarie, le strisce rimanenti devono essere sigillare nuovamente in un foglio di alluminio insieme con il sacchetto di gel di silice conservati a 2...8 °C.

6.2. Tampone di Lavaggio (20x conc.)

Diluire il Tampone di Lavaggio 1+19; per esempio: 10 mL del Tampone di Lavaggio + 190 mL di acqua distillata. Il Tampone di Lavaggio diluito è stabile per 5 giorni a temperatura ambiente (20…25 °C). Se cristalli appaiono nel concentrato, riscaldare la soluzione a 37 °C per esempio in un bagnomaria. Mescolare bene prima della diluizione.

6.3. Soluzione Substrato TMB

La soluzione sta pronta all'uso e deve essere conservata a 2...8 °C, al riparo dalla luce. La soluzione deve essere incolore o potrebbe avere un leggero colore blu chiaro. Se il substrato diventa blu, potrebbe essere stato contaminato e non può essere utilizzato nel test.

7. PRELIEVO E PREPARAZIONE DEI CAMPIONI

Per questo test si prega di usare campioni di siero o plasma (citrato, eparina) umano. Se il test è fatto entro 5 giorni dal prelievo i campioni possono essere conservati tra 2...8 °C. Altrimenti devono essere aliquotati e congelati tra (-70…-20 °C). Se i campioni sono conservati congelati, mescolare bene i campioni scongelati prima del test. Evitare cicli ripetuti di congelamento/scongelamento.
L'inattivazione dei campioni per mezzo del calore non è raccomandata.

7.1. Diluizione dei campioni

Prima del test, diluire i campioni 1+100 con Tampone di Diluizione del Campione IgM. Per esempio, pipettare nelle provette 10 µL di campione + 1 mL di Tampone di Diluizione del Campione IgM e mescolare bene (Vortex).

8. PROCEDIMENTO

Leggere bene le istruzioni per l'uso **prima** di iniziare il teste. L'affidabilità dei risultati dipende dalla stretta aderenza le istruzioni per l'uso di prova come descritto. La seguente procedura è stata validata per l'esecuzione manuale. Per un'esecuzione su strumentazione automatica si consiglia di incrementare il numero di lavaggi de 3 a 5 volte e il volume della Tampone di Lavaggio da 300 a 350 µL per evitare effetti di lavaggio. Prestare attenzione al capitolo 12. Stabilire innanzitutto il piano di distribuzione e identificazione dei campioni e standards/controlli (è raccomandato determinare in duplicato) sullo schema della piastra fornito con il kit. Inserire i pozzetti necessari nel supporto.

Eseguire il test nell'ordine stabilito dalle istruzioni, senza ritardi.

Sul pipettaggio utilizzare puntali nuovi e puliti per ogni campione e standard/controllo.

Regolare l'incubatore a 37 ± 1 °C.

1. Pipettare 100 µL di standard/controllo e di campione diluito nei relativi pozzetti. Usare il pozzetto A1 per il Bianco-substrato.
2. Coprire i pozzetti con la pellicola adesiva, fornita nel kit.
3. **Incubare 1 ora ± 5 min a 37 ± 1°C.**
4. Al termine dell'incubazione, togliere la pellicola e aspirare il liquido dai pozzetti. Successivamente lavare i pozzetti tre volte con 300 µL di Tampone di Lavaggio. Evitare che la soluzione trabocchi dai pozzetti. L'intervallo tra il lavaggio e l'aspirazione deve essere > 5 sec. Dopo il lavaggio picchiettare delicatamente i pozzetti su una carta assorbente per togliere completamente il liquido, prima del passo successivo.

   Attenzione: Il lavaggio è una fase molto importante. Da lavaggio insufficiente risulta una bassa precisione e risultati falsi!
5. Pipettare 100 µL di Coniugato in tutti i pozzetti, escludendo quello con il Bianco-substrato (Blank) A1.
6. **Incubare per 30 min a temperatura ambiente (20...25 °C).** Non esporre a fonti di luce diretta.
7. Ripetere il lavaggio secondo punto 4.
8. Pipettare 100 µL di Soluzione Substrato TMB in tutti i pozzetti.
9. **Incubare precisamente per 15 min a temperatura ambiente (20...25 °C) al buio.** Un colore blu verifica a causa della reazione enzimatica.
10. Pipettare 100 µL di Soluzione Bloccante in tutti i pozzetti, nello stesso ordine della Soluzione Substrato TMB, in tal modo un cambiamento di colore dal blu al giallo avviene.
11. Misurare l'assorbanza a 450/620 nm entro 30 min dopo l'aggiunta della Soluzione Bloccante.

8.1. Misurazione

Regolare il fotometro per le Piastre di Microtitolazione ELISA **a zero** usando il substrato-Bianco (Blank).

Se, per motivi tecnici, non è possibile regolare il fotometro per le Piastre di Microtitolazione a zero usando il Bianco-substrato, il valore de assorbanza de questo deve essere sottratto dai valori dell'assorbanza da tutti i valori delle altre assorbanze per ottenere risultati affidabili!

Misurare l'assorbanza di tutti i pozzetti a **450 nm** e inserire tutti i valori misurati nello schema della piastra.

È raccomandato fare le misurazioni delle onde **bichrome** (due colori). Utilizzando la lunghezza d'onda de 620 nm come misura di riferimento.

Dove sono state misurate in doppio, calcolare **la media delle assorbanze**.

9. RISULTATI

9.1. Validazione del test

Affinché un test possa essere considerato valido, le presenti Istruzioni per l'uso devono essere rigorosamente seguite e devono essere soddisfatti i seguenti criteri:

- **Substrato Bianco (Blank)**: Valore di assorbanza **< 0,100**
- **Controllo Negativo**: Valore di assorbanza **< 0,200 e < Cut-off**
- **Controllo Cut-off**: Valore di assorbanza **0,150 – 1,300**
- **Controllo Positivo**: Valore di assorbanza **> Cut-off**

Se non sono soddisfatti questi criteri, il test non è valido e deve essere ripetuto.

9.2. Calcolo dei risultati

Il Cut-off è la media dei valori di assorbanza dei Controlli Cut-off.

Esempio: Valore di assorbanza del Controllo Cut-off 0,44 + valore di assorbanza del Controllo Cut-off 0,42 = 0,86/2 = 0,43
Cut-off = 0,43

9.2.1. Risultati in unità [NTU]

$$\frac{\text{Assorbanza media del campione x 10}}{\text{Cut-off}} = \text{[unità NovaTec = NTU]}$$

Esempio: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretazione dei risultati

Cut-off	10 NTU	-
Positivo	> 11 NTU	Anticorpi contro il patogeno sono presenti. C'è stato un contatto con l'antigene (patogeno resp. vaccino).
Zona grigia	9 – 11 NTU	Anticorpi contro il patogeno non è stato possibile rilevare chiaramente. Si consiglia di ripetere il test con un nuovo campione in 2-4 settimane. Se il risultato è nuovamente nella zona grigia, il campione viene giudicato come **negativo**.
Negativo	< 9 NTU	Il campione non contiene anticorpi contro il patogeno. Un precedente contatto con l'antigene (patogeno resp. vaccino) è improbabile.
La diagnosi di una malattia infettiva non deve essere fatta soltanto sulla risultanza di un unico test. È importante considerare anche l'anamnesi ed i sintomi del paziente. I risultati del test da pazienti immunosoppressi e neonati hanno un valore limitato.		

9.3.1. Isotipi degli anticorpi e Stato dell'infezione

Sierologia	Significato
IgM	Caratteristica della risposta primaria dell'anticorpo Alto titolo IgM con basso titolo IgG: →suggerisce una infezione molto recente o acuta Raro: → IgM persistente
IgG	Caratteristica della risposta secondaria dell'anticorpo Può persistere per diversi anni Alto titolo IgG con basso titolo IgM: → può indicare un'infezione passata
IgA	Sono prodotte a livello delle mucose in tutto il corpo (⇒ barriera protettiva) Solitamente sono prodotte all'inizio dell'infezione

10. CARATTERISTICHE DEL TEST

I risultati si riferiscono al gruppo di campioni investigato; questi non sono specifiche garantite.

Per ulteriori informazioni su caratteristiche del test, si prega, di contattare NovaTec Immundiagnostica GmbH.

10.1. Precisione

Intradosaggio	**n**	**Media (E)**	**CV (%)**
#1	24	0,526	5,02
#2	24	0,905	6,20
#3	24	1,074	6,34
Interdosaggio	**n**	**Media (NTU)**	**CV (%)**
#1	12	19,56	7,08
#2	12	18,16	13,13
#3	12	5,28	7,31

10.2. Specificità diagnostica

La specificità diagnostica è la probabilità del test di fornire un risultato negativo in assenza di analita specifici.
La specificità diagnostica è 99,29% (95% intervallo di confidenza: 96,11% - 99,98%).

10.3. Sensibilità diagnostica

La sensibilità diagnostica è la probabilità del test di fornire un risultato positivo alla presenza di analita specifici.
La sensibilità diagnostica è 100% (95% intervallo di confidenza: 95,01% - 100%).

10.4. Possibili interferenze

Campioni emolitici, lipidici et itterici contenenti fino a 10 mg/mL di emoglobina, 5 mg/mL di trigliceridi e 0,5 mg/mL di bilirubina non hanno presentato fenomeni d'interferenza nel presente test.

10.5. Reattività crociata

L'investigazione di un gruppo di campioni con attività di anticorpi contro parametri potenzialmente interferenti non ha rivelato alcuna evidenza di risultati falsamente positivi dovuto a reattività crociata.

11. LIMITAZIONI

Una contaminazione da microorganismi o ripetuti cicli di congelamento-scongelamento possono alterare i valori delle assorbanze.

12. PRECAUZIONI E AVVERTENZE

- La procedura analitica, le informazioni, le precauzioni e le avvertenze contenute nelle istruzioni per l'uso devono essere seguite scrupolosamente. L'uso dei kit con analizzatori e attrezzature similari deve essere previamente convalidato. Qualunque cambiamento nello scopo, nel progetto, nella composizione o struttura e nella procedura analitica, così come qualunque uso dei kit in associazione ad altri prodotti non approvati dal produttore non è autorizzato; l'utilizzatore stesso è responsabile di questi eventuali cambiamenti. Il produttore non è responsabile per falsi risultati e incidenti che possano essere causati da queste ragioni. Il produttore non è responsabile per qualunque risultato ottenuto attraverso esame visivo dei campioni dei pazienti.
- Solo per uso diagnostico in-vitro.
- Tutti i materiali di origine umana o animale devono essere considerati potenzialmente contagiosi e infettivi.
- Tutti gli elementi di origine umana sono stati trovati non reattivi con Anti-HIV-Ab, Anti-HCV-Ab e HBsAg.
- Non scambiare reagenti e Piastre di Microtitolazione di lotti diversi.
- Non utilizzare reagenti d'altri produttori insieme con i reagenti di questo kit.
- Non usare dopo la data di scadenza.
- Utilizzare soltanto punte per pipette, distributori, e articoli da laboratorio puliti.
- Non scambiare i tappi dei flaconi, per evitare contaminazione crociata.
- Richiudere i flaconi immediatamente dopo l'uso per evitare la vaporizzazione e contaminazione.
- Una volta aperti e dopo relativo stoccaggio verificare i reagenti per una loro eventuale contaminazione prima dell'uso.
- Per evitare contaminazioni crociate e risultati erroneamente alti pipettare i campioni e reagenti con molta precisione nei pozzetti senza spruzzi.
- L'ELISA è progettato solo per il personale qualificato che segue le norme di buona pratica di laboratorio (Good Laboratory Practice, GLP).
- Per un ulteriore controllo di qualità interno ogni laboratorio dovrebbe inoltre utilizzare campioni noti.

12.1. Nota di sicurezza per i reagenti contenenti sostanze pericolose

I reagenti possono contenere CMIT/MIT (3:1) o MIT (vedi capitolo 4.1)
Pertanto, si applicano le seguenti indicazioni di pericolo e le consigli di prudenza.

Attenzione

H317	Può provocare una reazione allergica cutanea.
P261	Evitare di respirare gli aerosol.
P280	Indossare guanti/ indumenti protettivi.
P302+P352	IN CASO DI CONTATTO CON LA PELLE: lavare abbondantemente con sapone acqua.
P333+P313	In caso di irritazione o eruzione della pelle: consultare un medico.
P362+P364	Togliere tutti gli indumenti contaminati e lavarli prima di indossarli nuovamente.

Ulteriori informazioni sono disponibili nella scheda di dati di sicurezza.

12.2. Smaltimento

In genere tutte le sostanze chimiche sono considerati rifiuti pericolosi. Lo smaltimento è regolato da leggi nazionali. Per ulteriori informazioni contattare l'autorità locale.

13. INFORMAZIONI PER GLI ORDINI

Numero del prodotto:	MYCM0350	Mycoplasma pneumoniae IgM ELISA (96 determinazioni)

ESPAÑOL

1. INTRODUCCIÓN

Las Mycoplasma son procariotas con la caracteristica principal de no tener pared celular. Por esta facultad se forma la nueva clase de los “Mollicutes” (mollis: suave, cutis: piel). La familia de los Mycoplasmataceae se divide en los géneros Mycoplasma y Ureaplasma. En los Mycoplasma se conocen más de 80 especies dentro de las más importantes como patógenos para el hombre son M. pneumoniae, M. hominis y M. genitalis. Aparte de estos patógenos obligatorios existen varias especies que son facultativo patógeno (M. orale, M. salivarium, M. faucium, M. buccale).

M. pneumoniae produce infecciones del tracto respiratoiro (neumonías atípicas), M. hominis y M. genitalis infectan al tracto urogenital. M. pneumoniae coloniza la traquea y los bronquios destruyendo el epitelio sin entrar en el tejido peribronquial. Las infiltraciones en el tejido peribronquial son probablemente reacciones fuertes del sistema inmunitario con presencia de linfocitos, células plasma y macrófagos. Muchas veces se muestran fenómenos autoinmunitarios en forma de la formación de anticuerpos con reacciones cruzadas con tejidos corporales. Después de un período de incubación medio de 3 semanas se producen en el 75% de los casos infecciones gripales fuertes con faringitis o traqueobronquitis. Del 5 al 25 % de los casos solamente de desarolla una neumonía atípica que comienza con cansancio, dolores de cabeza, fiebre y tos pertinante.

El M. pneumoniae no pertenece a la flora normal del hombre. Las Mycoplasma son de manifestación mundial y se transmiten con alta contagiosidad por el aire y por gotats de fluido contaminado. El grupo más afectado son los jovenes de 5 a 20 años. Las infecciones dentro de una familia o en comunidades se explican por la alta contagiosidad y el modo de infección. Son comunes las reinfecciones.

Especies	**Enfermedad**	**Síntomas (p.ej.)**	**Vía de transmisión**
M. pneumoniae	Enfermedad respiratoria por Mycoplasma pneumoniae	Fiebre, dolor de cabeza y tos persistente Enfermedad respiratoria, de infección asintomática a resfriados, faringitis, bronquitis, krupp, traqueobronquitis, pneumonitis y neumonía atípica primaria	aerógena, por gotitas de fluido contaminado

La infección o la presencia de un patógeno puede identificarse mediante:

- Microscopia
- Serología: p.ej. ELISA

2. USO PREVISTO

El enzimoinmunoensayo Mycoplasma pneumoniae IgM ELISA se utiliza para la determinación cualitativa de anticuerpos IgM específicos contra Mycoplasma pneumoniae en suero o plasma (citrato, heparina) humano.

3. PRINCIPIO DEL ENSAYO

La determinación inmunoenzimática cualitativa de anticuerpos específicos se basa en la técnica ELISA (Enzyme-linked Immunosorbent Assay).
Las Placas de Microtitulación están recubiertas con antígenos específicos unen a los anticuerpos de la muestra. Después de lavar los pocillos para eliminar todo el material de muestra no unida, el conjugado de peroxidasa de rábano (HRP) se añade. Este conjugado se une a los anticuerpos capturados. En una segunda etapa de lavado se retira el conjugado no unido. El complejo inmune formado por el conjugado unido se visualiza añadiendo substrato tetrametilbencidina (TMB), que da un producto de reacción azul.
La intensidad de este producto es proporcional a la cantidad de anticuerpos específicos en la muestra. se añade ácido sulfúrico para detener la reacción. Esto produce un cambio de color de azul a amarillo. La extinción a 450/620 nm se mide con un fotómetro de Placa de Microtitulación ELISA.

4. MATERIALES

4.1. Reactivos suministrados

- **Placa de Microtitulación:** 12 tiras de 8 pocillos rompibles, recubiertos con antígenos de Mycoplasma pneumoniae, en bolsa de aluminio.
- **Tampón de Dilución de Muestras IgM:** 1 botella de 100 mL de solución de tampón de fosfato (10 mM) para diluir la muestra; pH 7,2 ± 0,2; anti-humana IgG (RF Absorbente); color verde; listo para ser utilizado; tapa blanca; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solución de Parada:** 1 botella de 15 mL de ácido sulfúrico, 0,2 mol/L, listo para ser utilizado; tapa roja.
- **Tampón de Lavado (20x conc.):** 1 botella de 50 mL de una solución de tampón de fosfato 20x concentrado (0,2 M) para lavar los pocillos; pH 7,2 ± 0,2; tapa blanca.
- **Conjugado:** 1 botella de 20 mL de conjugado de anticuerpos IgM anti-humano con peroxidasa en tampón de fosfato (10 mM); color rojo; tapa negra; listo para ser utilizado.
- **Solución de Sustrato de TMB:** 1 botella de 15 mL 3,3',5,5'-tetrametilbenzindina (TMB), < 0,1 %; listo para ser utilizado; tapa amarilla.
- **Control Positivo:** 1 botella de 2 mL control; color amarillo; tapa roja; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Cut-off:** 1 botella de 3 mL control; color amarillo; tapa verde; listo para ser utilizado; ≤ 0,02% (v/v) MIT.
- **Control Negativo:** 1 botella de 2 mL control; color amarillo; tapa azul; listo para ser utilizado; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Para indicaciones de peligro y consejos de prudencia consulte el cap. 12.1.
Para sustancias potencialmente peligrosas por favor revise la ficha de datos de seguridad.

4.2. Accesorios suministrados

- 1 lámina autoadhesiva
- 1 instrucciones de uso
- 1 esquema de la placa

4.3. Materiales e instrumentos necesarios

- Fotómetro de Placa de Microtitulación con filtros de 450/620 nm
- Incubadora 37 °C
- Dispositivo de lavado manual o automático de Placa de Microtitulación
- Micropipetas para uso de (10-1000 µL)
- Mezcladora Vortex
- Agua destilada
- Tubos de plástico desechables

5. ESTABILIDAD Y ALMACENAJE

Almacene el kit a 2...8 °C. Los reactivos abiertos son estables hasta la fecha de caducidad indicada en la etiqueta cuando se almacena a 2...8 °C.

6. PREPARACIÓN DE LOS REACTIVOS

Es muy importante llevar todos los reactivos y las muestras a temperatura ambiente (20...25 °C) y mezclarlos antes de ser utilizados!

6.1. Placa de Microtitulación

Las tiras rompibles están recubiertas con antígeno de Mycoplasma pneumoniae. Inmediatamente después de la eliminación de las tiras, las tiras restantes deben sellarse de nuevo en el papel de aluminio junto con la bolsita di dióxio de silicio y almacenar a 2...8 °C.

6.2. Tampón de Lavado (20x conc.)

Diluir el Tampón de Lavado 1+19; por ejemplo 10 mL del Tampón de Lavado + 190 mL de agua destilada. El Tampón de Lavado diluido es estable durante 5 días a temperatura ambiente (20…25 °C). En caso de aparecer cristales en el concentrado, calentar la solución a 37 °C, por ejemplo, en un baño María. Mezclar bien antes de la dilución.

6.3. Solución de Sustrato de TMB

La solución está lista para su uso y debe almacenarse a 2...8 °C, protegida de la luz. La solución debe ser incolora o podría tener un color ligeramente azul claro. Si el sustrato se convierte en azul, es posible que haya sido contaminado y no puede ser utilizado en el ensayo.

7. TOMA Y PREPARACIÓN DE LAS MUESTRAS

Usar muestras de suero o plasma (citrato, heparina) humano. Si el ensayo se realiza dentro de 5 días después de la toma de sangre, las muestras pueden ser almacenadas a 2...8 °C, en caso contrario deben ser alicuotadas y almacenadas congeladas (-70...-20 °C). Agitar bien las muestras descongeladas antes de diluirlas. Evitar congelaciones y descongelaciones repetidas. No se recomienda la inactivación por calor de las muestras.

7.1. Dilución de las muestras

Antes del ensayo, las muestras tienen que estar diluidas en relación 1 + 100 con el Tampón de Dilución de Muestras IgM, p. e. 10 µL de la muestra con 1 mL de Tampón de Dilución de Muestras IgM, mezclar bien con la mezcladora Vortex.

8. PROCEDIMIENTO

Por favor, leer cuidadosamente las instrucciones de uso del ensayo **antes** de realizarlo. Para el buen funcionamiento de la técnica es necesario seguir las instrucciones. El siguiente procedimiento es válido solamente para el método manual. Si se realiza el ensayo en los sistemas automáticos de ELISA es aconsejable elevar el número de lavados de tres hasta cinco veces y el volumen de Tampón de Lavado de 300 µL a 350 µL para excluir efectos de lavado. Preste atención al capítulo 12. Antes de comenzar, especificar exactamente la repartición y posición de las muestras y de los estándares/controles (se recomienda determinar en duplicado) en el esquema de la placa suministrada. Usar la cantidad necesaria de tiras o pocillos e insertarlos en el soporte.

Realizar el ensayo en el orden indicado y sin retraso.

Para cada paso de pipeteado en los estándares/controles y en las muestras, usar siempre puntas de pipeta de un solo uso.

Graduar la incubadora a 37 ± 1°C.

1. Pipetear 100 µL de estándares/controles y muestras en los pocillos respectivos. Dejar el pocillo A1 para el blanco.
2. Recubrir las tiras con los autoadhesivos suministrados.
3. **Incubar 1 h ± 5 min a 37 ± 1°C.**
4. Después de la incubación, retirar el autoadhesivo, aspirar el líquido de la tira y lavarla tres veces con 300 µL del Tampón de Lavado. Evitar el rebosamiento de los pocillos. El intervalo entre lavado y aspiración debe ser > 5 segundos. Para sacar el líquido restante de las tiras, es conveniente sacudirlas sobre papel absorbente.

   Nota: El lavado es muy importante! Un mal lavado insuficiente provoca una baja precisión y resultados falsamente elevados!
5. Pipetar 100 µL de conjugado en cada pocillo con excepción del blanco substrato A1.
6. **Incubar 30 min a la temperatura ambiente (20…25 °C).** Evitar la luz solar directa.
7. Repetir el lavado como en el paso numero 4.
8. Pipetar 100 µL de la Solución de Sustrato de TMB en todos los pocillos.
9. **Incubar exactamente 15 min en oscuridad a temperatura ambiente (20...25 °C).** Un color azul se produce en las muestras positivas debido a la reacción enzimática.
10. Pipetear en todos los pocillos 100 µL de la Solución de Parada en el mismo orden y mismo intervalo de tiempo como con el Solución de Sustrato de TMB, por lo tanto un cambio de color de azul a amarillo se produce.
11. Medir la extinción con 450/620 nm en un periodo de 30 min después de añadir la Solución de Parada.

8.1. Medición

Ajustar el fotómetro de Placa de Microtitulación ELISA al cero utilizando el Blanco.

Si por razones técnicas el fotómetro de Placa de Microtitulación de ELISA no se puede ajustar a cero utilizando el Blanco, el valor de la absorbancia de este debe ser sustraído de los demás valores de absorbancia medidos con el fin de obtener resultados fiables!

Medir la **extinción** de todos los pocillos con **450 nm** y anotar los resultados de los estándares/controles y de las muestras en el esquema de la placa.

Es aconsejable realizar la medición **bicromática** a una longitud de onda de referencia de 620 nm.

Si se efectuaron análisis en duplicado o múltiples, hay que calcular **el promedio de los valores de extinción** de los pocillos correspondientes.

9. CÁLCULO DE LOS RESULTADOS

9.1. Criterios de validez del ensayo

Para que un ensayo se considere válido, deben seguirse estrictamente las presentes instrucciones de uso y deben cumplirse los siguientes criterios:

- **Blanco:** valor de la extinción **< 0,100**
- **Control Negativo:** valor de la extinción **< 0,200 y < Cut-off**
- **Control Cut-off:** valor de la extinción **0,150 – 1,300**
- **Control Positivo:** valor de la extinción **> Cut-off**

Si estos criterios no se cumplen, la prueba no es váida y deberá repetirse.

9.2. Cálculo del valor de la medición

El Cut-off se obtiene de los valores de la extinción de lo Control Cut-off.

Ejemplo: 0,42 OD Control Cut-off + 0,44 OD Control Cut-off = 0,86:2 = 0,43

Cut-off = 0,43

9.2.1. Resultados en unidades [NTU]

$$\frac{\text{Promedio valor de la extinción de la muestra x 10}}{\text{Cut-off}} = \text{[NovaTec-unidades = NTU]}$$

Ejemplo: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretación de los resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Los anticuerpos contra el patógeno están presentes. Ha producido un contacto con el antígeno (patógeno resp. vacuna).
Zona intermedia	9 – 11 NTU	Los anticuerpos contra el patógeno no se pudieron detectar claramente. Se recomienda repetir la prueba con una muestra fresca en 2 a 4 semanas. Si el resultado es de nuevo en la zona intermedia, la muestra se considera como **negativa**.
Negativo	< 9 NTU	La muestra no contiene anticuerpos contra el patógeno. Un contacto previo con el antígeno (patógeno resp. vacuna) es poco probable.
El diagnóstico de una infección no solamente se debe basar en el resultado del ensayo. Es necesario considerar la anamnésis y la sintomatología del paciente junto al resultado serológico. Estos resultados sólo tienen valor restringido en pacientes inmunodeprimidos o en neonatos.		

9.3.1. Isotipos de anticuerpo y Estado de la Infección

Serología	Significado
IgM	Característica de la respuesta primaria del anticuerpo Alto título de IgM con bajo título de IgG → sugieren una infección muy reciente o aguda Raras: → persistente IgM
IgG	Característica de la respuesta secundaria del anticuerpo Pueden persistir por varios años El alto título de IgG con bajo título de IgM: → pueden indicar una infección pasada
IgA	Producida en el revestimiento mucoso en todo el cuerpo (⇒ Barrera Protectora) Usualmente producida tempranamente en el transcurso de la infección

10. CARACTERÍSTICAS DEL ENSAYO

Los resultados están basados en el grupo de pruebas investigado; no se trata de especificaciones garantizadas.

Para obtener más información sobre las características del ensayo, por favor, entre en contacto NovaTec Immundiagnostica GmbH.

10.1. Precisión

Intra ensayo	**n**	**Promedia (E)**	**CV (%)**
#1	24	0,526	5,02
#2	24	0,905	6,20
#3	24	1,074	6,34
Inter ensayo	**n**	**Promedia (NTU)**	**CV (%)**
#1	12	19,56	7,08
#2	12	18,16	13,13
#3	12	5,28	7,31

10.2. Especificad diagnóstica

La especificidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado negativo en ausencia del analítico específico. Es 99,29% (95% Intervalo de confianza: 96,11% - 99,98%).

10.3. Sensibilidad de diagnóstico

La sensibilidad del ensayo se define como la probabilidad que tiene el ensayo de dar un resultado positivo en presencia del analítico específico. Es 100% (95% Intervalo de confianza: 95,01% - 100%).

10.4. Interferencias

Las muestras lipémicas, ictéricas e hemolíticas no mostraron interferencias con este equipo ELISA hasta una concentración de 5 mg/mL para triglicéridos, de 0,5 mg/mL para bilirrubina y de 10 mg/mL hemoglobina.

10.5. Reactividad cruzada

Pruebas realizadas con un panel de muestras con distinta actividad de anticuerpos para estudiar parámetros de reactividad no dieron falsos positivos debidos a reactividad cruzada.

11. LIMITACIONES DEL ENSAYO

Una contaminación de las muestras con bacterias, o una congelación y descongelación repetida pueden producir cambios en los valores de la extinción.

12. PRECAUCIONES Y ADVERTENCIAS

- El procedimiento, la información, las precauciones y los avisos de las instrucciones de uso han de ser seguidas estrictamente. La utilización de equipos con analizadores y equipamiento similar tiene que ser validada. No se autorizan cambios en el diseño, composición y procedimiento, así como cualquier utilización en combinación con otros productos no aprobados por el fabricante; el usuario debe hacerse responsable de estos cambios. El fabricante no responderá ante falsos resultados e incidentes debidos a estas razones. El fabricante no responderá ante cualquier resultado por análisis visual de las muestras de los pacientes.
- Solo para diagnostico in vitro.
- Todos los materiales de origen humano o animal deberán ser considerados y tratados como potencialmente infecciosos.
- Todos los componentes de origen humano han sido examinados y resultaron no reactivos a anticuerpos contra el VIH, VHC y HbsAG.
- No intercambiar reactivos y Placa de Microtitulación de cargas diferentes.
- No usar reactivos de otro fabricante para este ensayo.
- No usar después de la fecha de caducidad.
- Sólo usar recambios de pipetas, dispensadores y materiales de laboratorio limpios.
- No intercambiar las tapas de los diferentes reactivos, para evitar la contaminación cruzada.
- Para evitar la evaporación y una contaminación microbiana, cierre inmediatamente las botellas después de usarlas.
- Después de abrirlas y posterior almacenaje, asegurarse de que no existe contaminación microbiana antes de seguir usándolas.
- Para evitar contaminaciones cruzadas y resultados erróneamente aumentados, Pipetear cuidadosamente las muestras y los reactivos en los pocillos sin salpicar.
- El ELISA sólo está diseñado para personal cualificado siguiendo las normas de buenas prácticas de laboratorio (Good Laboratory Practice,GLP).
- Para un mayor control de calidad interno, cada laboratorio deberá utilizar además muestras conocidas.

12.1. Nota de seguridad para los reactivos que contienen sustancias peligrosas

Los reactivos pueden contener CMIT/MIT (3:1) o MIT (consulte el cap. 4.1)
Por lo tanto, se aplican las indicaciones de peligro y consejos de prudencia.

Atención

H317	Puede provocar una reacción alérgica en la piel.
P261	Evitar respirar el aerosol.
P280	Llevar guantes/ prendas de protección.
P302+P352	EN CASO DE CONTACTO CON LA PIEL: Lavar con abundante jabón agua.
P333+P313	En caso de irritación o erupción cutánea: Consultar a un médico.
P362+P364	Quitar las prendas contaminadas y lavarlas antes de volver a usarlas.

Se puede encontrar más información en la ficha de datos de seguridad.

12.2. Indicaciones para la eliminación de residuos

Por regla general, los productos químicos y las preparaciones son residuos peligrosos. Su eliminación esta sometida a las leyes y los decretos nacionales sobre la eliminación de residuos. Las autoridades informan sobre la eliminación de residuos peligroso.

13. INFORMACIONES PARA PEDIDOS

N° del producto:	MYCM0350	Mycoplasma pneumoniae IgM ELISA (96 determinaciones)

PORTUGUÊS

1. INTRODUÇÃO

Os micoplasmas pertencem à classe Mollicutes composta por três famílias e quatro géneros distintos, um dos quais é o Mycoplasma com mais de 60 espécies. Os Micoplasmas são os menores organismos de vida livre conhecidos (300 a 500 nm de diâmetro) e, ao contrário das bactérias normais não possuem uma parede celular. Os Micoplasmas são parasitas extracelulares, especialmente nas membranas mucosas, que podem causar infecções em humanos, animais, plantas e culturas celulares. O Mycoplasma pneumoniae é essencialmente um agente patogénico respiratório (obrigatório) em humanos envolvendo a nasofaringe, garganta, traqueia, brônquios, bronquíolos e alvéolos. Outros Micoplasmas, M. buccale, M. faucium, M. orale e M. salivarium são comensais na cavidade oral. O Mycoplasma hominis e o Ureaplasma urealyticum habitam principalmente o trato genital e podem actuar como invasores oportunistas. O M. pneumoniae é de longe o agente patogénico mais importante deste grupo. A infecção por M. pneumoniae ocorre em todo o mundo, a sua epidemiologia tem sido estudada principalmente no E.U.A., Europa e Japão. As infecções são endémicas nas grandes áreas urbanas, e são observados aumentos da epidemia em intervalos variáveis. Foi estimado que o M. pneumoniae seja a causa de 15-20% de todas as pneumonias, a taxa é mais elevada em crianças e adultos jovens. 74% das infecções por M. pneumoniae são assintomáticas, a reinfecção pode ocorrer. A imunidade adquirida naturalmente à infecção por M. pneumoniae parece ser de duração limitada (2-3 anos).

Espécies	Doença	Sintomas (p.ex.)	Via de transmissão
M. pneumoniae	Doença respiratória Mycoplasma pneumonia	Febre, cefaléia e tosse persistente Doença respiratória: de infecção assintomática ao resfriados, faringite, bronquite, crup, traqueobronquite, pneumonite e pneumonia primária atípica	Por via aérea, a transmissão do vírus ocorre por infecção por gotículas;

Infecção ou presença de patógeno pode ser identificada por:

- Microscopia
- Serologia: p. ex. ELISA

2. UTILIZAÇÃO PRETENDIDA

O kit Mycoplasma pneumoniae IgM ELISA destina-se à determinação qualitativa de anticorpos da classe IgM contra Mycoplasma pneumoniae no soro ou plasma (citrato, heparina) humanos.

3. PRINCÍPIO DO ENSAIO

A determinação imunoenzimática qualitativa de anticorpos específicos é baseado na técnica de ELISA (do inglês Enzyme-linked Immunosorbent Assay).
As Placas de Microtitulação são revestidas com antigénios específicos que se ligam os anticorpos correspondentes da amostra. Após lavagem dos poços, para remover todo o material de amostra não ligado, um conjugado de peroxidase de rábano (HRP) é adicionado. Este conjugado se liga aos anticorpos capturados. Num segundo passo de lavagem o conjugado não ligado é removido. O complexo imune formado pelo conjugado ligado é visualizado por adição de substrato de tetrametilbenzidina (TMB), o que dá um produto de reacção azul.
A intensidade deste produto é proporcional à quantidade de anticorpos específicos da amostra. O ácido sulfúrico é adicionado para parar a reacção. Isso produz uma mudança de cor de azul para amarelo.
Absorvância a 450/620 nm é lida utilizando um fotômetro de Placa de Microtitulação ELISA.

4. MATERIAIS

4.1. Reagentes fornecidos

- **Placa de Microtitulação:** 12 tiras de 8 poços, destacáveis e quebráveis, revestidas com antigénio de Mycoplasma pneumoniae, em bolsas de folha de alumínio com fecho.
- **Tampão de Diluição de Amostra IgM:** 1 frasco contendo 100 mL de tampão fosfato (10 mM) para diluição da amostra, pH 7,2 ± 0,2; anti-humana IgG (RF Absorbent); de cor verde; pronto a usar; tampa branca; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).
- **Solução de Bloqueio:** 1 frasco contendo 15 mL ácido sulfúrico; 0,2 mol/L; pronto a usar; tampa vermelha.
- **Tampão de Lavagem (conc. 20x):** 1 frasco contendo 50 mL de um tampão fosfato (0,2 M); concentrado 20 vezes (pH 7,2 ± 0,2) para a lavagem dos poços; tampa branca.
- **Conjugado:** 1 frasco contendo 20 mL de anticorpo para IgM humana marcados com peroxidase no tampão fosfato (10 mM); de cor vermelho, pronto a usar; tampa preta.
- **Solução Substrato TMB:** 1 frasco contendo 15 mL de 3,3',5,5'-tetrametilbenzidina (TMB), < 0,1 %; pronto a usar; tampa amarela.
- **Controle Positivo:** 1 frasco contendo 2 mL controle; de cor amarela; pronto a usar; tampa vermelha; ≤ 0,02% (v/v) MIT.
- **Controle Cut-off:** 1 frasco contendo 3 mL controle; de cor amarela; pronto a usar; tampa verde; ≤ 0,02% (v/v) MIT.
- **Controle Negativo:** 1 frasco contendo 2 mL controle; de cor amarela; pronto a usar; tampa azul; ≤ 0,0015% (v/v) CMIT/ MIT (3:1).

Para advertências de perigo e recomendaçaões de prudência ver capitulo 12.1.
Para substâncias potencialmente perigosas verifique a ficha de dados de segurança.

4.2. Materiais fornecidos

- 1 Película de cobertura
- 1 Instruções de uso
- 1 Layout da placa

4.3. Materiais e Equipamento necessários

- Fotômetro de Placa de Microtitulação ELISA, equipado para a medição da absorvância a 450/620 nm
- Incubadora 37 °C
- Equipamento manual ou automático para a lavagem de Placas de Microtitulação
- Pipetas para dispensar volumes entre 10 e 1000 µL
- Agitador de tubos tipo Vortex
- Água destilada
- Tubos descartáveis

5. ESTABILIDADE E ARMAZENAMENTO

Armazene o kit a 2...8 °C. Os reagentes abertos são estáveis até o prazo de validade impresso no rótulo quando armazenado a 2...8 °C.

6. PREPARAÇÃO DOS REAGENTES

É muito importante deixar todos os reagentes e amostras estabilizar à temperatura ambiente (20...25 °C) misturá-los antes de iniciar o teste!

6.1. Placa de Microtitulação

As tiras quebraveis são revestidas com antigénio Mycoplasma pneumoniae. Imediatamente após a remoção das tiras necessarias, as tiras restantes devem ser lacradas de novo na folha de alumínio juntamente com o saquinho de silicio fornecido e armazenadar a 2...8 °C.

6.2. Tampão de Lavagem (conc. 20x)

Diluir o Tampão de Lavagem 1+19; por exemplo. 10 mL do Tampão de Lavagem + 190 mL de água destilada. O Tampão de Lavagem diluído é estavel durante 5 dias à temperatura ambiente (20…25 °C). Caso apareça cristais no concentrado, aquecer a solução a 37 °C por exemplo, em banho Maria. Misture bem antes da diluição.

6.3. Solução Substrato TMB

A solução está pronta para uso e tem de ser armazenada à 2...8 °C, protegida da luz. A solução deve ser incolor ou poderia ter uma ligeira coloração azul claro. Se o substrato se transforma em azul, pode ter sido contaminado e não pode ser usado no teste.

7. COLHEITA E PREPARAÇÃO DAS AMOSTRAS

Usar com este ensaio amostras de soro ou plasma (citrato, heparina) humanos. Se o ensaio for realizado dentro de 5 dias após colheita da amostra, o espécime deve ser mantido a 2…8 °C; caso contrário devem ser alicotadas e armazenadas congeladas (-70...-20 °C). Se as amostras forem armazenadas congeladas, misturar bem as amostras descongeladas antes de testar. Evitar congelar e descongelar repetidamente.
Não é recomendada a inactivação por calor das amostras.

7.1. Diluição das amostras

Antes de testar todas as amostras devem ser diluídas 1 + 100 com Tampão de Diluição de Amostra IgM. Dispensar 10 µL de amostra e 1 mL de Tampão de Diluição de Amostra IgM em tubos para obter uma diluição 1 + 100 e misturar meticulosamente com um vortex.

8. PROCEDIMENTO DO ENSAIO

Por favor, ler atentamente as instruções de uso **antes** de realizar o teste. A fiabilidade dos resultados depende da adesão estrita ao as instruções de uso, conforme descritas. O procedimento de ensaio a seguir está validado apenas para o procedimento manual. Se o teste for realizado em sistemas automáticos para teste ELISA é recomendável aumentar os passos de lavagem de três até cinco e o volume do Tampão de Lavagem de 300 µL para 350 µL para evitar efeitos de lavagem. Preste atenção ao capítulo 12. Antes de iniciar o teste, o plano de distribuição e identificação de todas as amostras e calibradores/controles (é recomendado determinar em duplicidade) deve ser cuidadosamente estabelecido no Layout da placa fornecida no kit. Seleccionar o número necessário de tiras ou poços e inserir os mesmos no suporte.

Realizar todas as etapas do teste na ordem indicada e sem atrasos significativos.

Na pipetagem deve ser utilizada uma ponta limpa e descartável para dispensar cada controle e amostra.

Ajustar a incubadora para 37 ± 1 °C.

1. Dispensar 100 µL dos calibradores/controles e das amostras diluídas nos poços respectivos. Deixar o poço A1 vazio para o branco substrato.
2. Cobrir os poços com a película fornecida no kit.
3. **Incubar durante 1 hora ± 5 min a 37 ± 1 °C.**
4. Quando terminar a incubação, remover a película, aspirar o conteúdo dos poços e lavar cada poço três vezes com 300 µL de Tampão de Lavagem. Evitar que os poços de reacção transbordem. O intervalo entre a lavagem e a aspiração deve ser > 5 seg. No final, retirar cuidadosamente o fluido restante batendo delicadamente as tiras sobre papel absorvente, antes da próxima etapa!

   Nota: A lavagem é muito importante! Lavagem insuficiente resulta em baixa precisão e falsos resultados.
5. Dispensar 100 µL de Conjugado em todos os poços, excepto no poço do Branco substrato A1.
6. **Incubar durante 30 min à temperatura ambiente (20...25°C).** Não expor diretamente à luz solar.
7. Repetir a etapa 4.
8. Dispensar 100 µL de Solução Substrato TMB em todos os poços.
9. **Incubar durante exactamente 15 min à temperatura ambiente (20...25°C) e no escuro.** A cor azul devido a uma reacção enzimática.
10. Dispensar 100 µL de Solução de Bloqueio em todos os poços, pela mesma ordem e com a mesma velocidade a que foi dispensada a Solução Substrato TMB,desse modo uma mudança de cor de azul para amarelo ocorre.
11. Medir a absorvância a 450/620 nm dentro de 30 min após a adição da Solução de Bloqueio.

8.1. Medição

Ajustar o fotômetro para Placa de Microtitulação ELISA **a zero** usando o **Branco substrato .**

Se - devido à razões técnicas – o fotômetro para Placa de Microtitulação ELISA não puder ser ajustado a zero usando o Branco substrato, valor da absorvância deste deve ser subtraido de todos os outros valores de absorvância medidos de forma a obter resultados fiáveis!

Medir a absorvância de todos os poços a **450 nm** e registar os valores da absorvância para cada calibrador/controle e amostra no Layout da placa.

É recomendado fazer a medição **dicromática** usando como referência um comprimento de onda de 620 nm.

Se determinações duplas foram realizadas, calcular **os valores médios de absorvância**.

9. RESULTADOS

9.1. Critérios de validação do ensaio

Para que um ensaio seja considerado válido, estas Instruções de Uso devem ser rigorosamente seguidas, e os seguintes critérios devem ser cumpridos:

- **Branco substrato**: Valor de Absorvância **< 0,100**
- **Controle Negativo**: Valor de Absorvância **< 0,200 e < Cut-off**
- **Controle Cut-off**: Valor de Absorvância **0,150 – 1,300**
- **Controle Positivo**: Valor de Absorvância **> Cut-off**

Se estes critérios não forem cumpridos, o teste não é válido e deve ser repetido.

9.2. Cálculo dos Resultados

O Cut-off é o valor médio da absorvância das determinações do Controle Cut-off.

Exemplo: Valor da absorvância do Controle Cut-off 0,42 + valor da absorvância do Controle Cut-off 0,44 = 0,86: 2 = 0,43
Cut-off = 0,43

9.2.1. Resultados em Unidades [NTU]

$$\frac{\text{Valor da absorvância (média) da amostra} \times 10}{\text{Cut-off}} = \text{[Unidades NovaTec = NTU]}$$

Exemplo: $\frac{1{,}591 \times 10}{0{,}43} = 37$ NTU

9.3. Interpretação dos Resultados

Cut-off	10 NTU	-
Positivo	> 11 NTU	Os anticorpos contra o agente patogênico estão presente. Houve um contacto com o antigénio (patógeno resp vacina).
Zona cinzenta	9 – 11 NTU	Os anticorpos contra o agente patogênico não puderam ser claramente detectados. Recomenda-se a repetir o teste com uma amostra fresca em 2 a 4 semanas. Se o resultado estiver novamente dentro da zona cinzenta, a amostra é julgada como **negativa**.
Negativo	< 9 NTU	A amostra não contém os anticorpos contra o agente patogênico. Um contato prévio com o antígeno (patógeno resp. vacina) é improvável.
O diagnóstico de uma doença infecciosa não deve ser estabelecido com base num único resultado do teste. Um diagnóstico preciso deve ter em consideração a história clínica, a sintomatologia bem como dados serológicos. Em paciente imunossuprimidos e recém-nascidos os dados serológicos têm apenas valor restrito.		

9.3.1. Isotipos de anticorpos e Estado da Infecção

Sorologia	Significado
IgM	Característica da resposta primária do anticorpo Alto título de IgM com baixo título de IgG: → sugere uma infecção muito recente ou aguda Raros: → persistente IgM
IgG	Característica da resposta secundária do anticorpo Podem persistir por vários anos Alto título de IgG com baixo título de IgM: → pode indicar uma infecção passada
IgA	Eles são produzidos a nível das mucosas em todo o corpo (⇒ barreira protectora) Geralmente são producidas no inicio infecção

10. CARACTERÍSTICAS DE DESEMPENHO ESPECÍFICAS

Os resultados referem-se aos grupos de amostras investigados; estas não são especificações garantidas.

Para mais informações sobre as características de desempenho específicas, por favor, entre em contato NovaTec Immundiagnostica GmbH.

10.1. Precisão

Intra ensaio	**n**	**Média (E)**	**CV (%)**
#1	24	0,526	5,02
#2	24	0,905	6,20
#3	24	1,074	6,34
Inter ensaio	**n**	**Média (NTU)**	**CV (%)**
#1	12	19,56	7,08
#2	12	18,16	13,13
#3	12	5,28	7,31

10.2. Especificidade Diagnóstica

A especificidade diagnóstica é definida como a probabilidade do ensaio ser negativo na ausência do analito específico.
É de 99,29% (95% Intervalo de confiança: 96,11% - 99,98%).

10.3. Sensibilidade Diagnóstica

A sensibilidade diagnóstica é definida como a probabilidade do ensaio ser positivo na presença do analito específico.
É de 100% (95% Intervalo de confiança: 95,01% - 100%).

10.4. Interferências

Não são observadas interferências com amostras hemolisadas, lipémicas ou ictéricas até uma concentração de hemoglobina de 10 mg/mL, de triglicerídeos de 5 mg/mL e de bilirrubina de 0,5 mg/mL.

10.5. Reacção cruzada

A investigação do painel de amostras com atividades de anticorpos em parâmetros com potencial de reação cruzada não revelou nenhuma evidencia de resultados falso-positivos devido a reações cruzadas.

11. LIMITAÇÕES DO PROCEDIMENTO

Contaminação bacteriana ou a repetição de ciclos de congelação-descongelação do espécime podem afectar os valores da absorvância.

12. PRECAUÇÕES E AVISOS

- O procedimento do teste, as informações, as precauções e avisos nas instruções para utilização têm de ser rigorosamente seguidas. O uso de kits de teste com analisadores e equipamento similar tem de ser validado. Qualquer alteração no desenho, composição e procedimento do teste bem como qualquer utilização em combinação com outros produtos não aprovados pelo fabricante não estão autorizados; o próprio utilizador é responsável por tais alterações. O fabricante não é legalmente responsável por resultados falsos e incidentes originados por estes motivos. O fabricante não é legalmente responsável por quaisquer resultados obtidos por análise visual das amostras dos pacientes.
- Apenas para uso no diagnóstico in-vitro.
- Todos os materiais de origem humana ou animal devem ser considerados e tratados como potencialmente infectantes.
- Todos os componentes de origem humana usados para a produção destes reagentes foram testados para anticorpos anti-HIV, anticorpos anti-HCV e HBsAg e foram considerados não-reactivos.
- Não trocar e/ou juntar reagentes ou Placa de Microtitulação de lotes de produção diferentes.
- Nenhuns reagentes de outros fabricantes devem ser usados juntamente com reagentes deste kit de teste.
- Não usar reagentes após a data de validade indicada no rótulo.
- Usar apenas pontas de pipeta, dispensadores e material de laboratório limpos.
- Não trocar as tampas dos frascos dos reagentes para evitar contaminação cruzada.
- Fechar firmemente os frascos dos reagentes imediatamente após a utilização para evitar evaporação e contaminação microbiana.
- Após a primeira abertura e armazenamento subsequente verificar se existe contaminação microbiana dos frascos do conjugado e dos calibradores/controles antes de utiliza-los novamente.
- Para evitar contaminação-cruzada e resultados falsamente elevados, pipetar as amostras dos pacientes e dispensar o reagentes precisamente nos poços sem salpicar.
- O ELISA é projetado apenas para pessoal qualificado seguindo os padrões de boas práticas de laboratório (Good Laboratory Practice, GLP).
- Para um controle de qualidade interno adicional cada laboratório deve utilizar amostras conhecidas.

12.1. Nota de segurança para reagentes que contenham substâncias perigosas

Os reagentes podem conter CMIT/MIT (3:1) or MIT (ver capitulo 4.1)
Portanto, as seguintes advertências de perigo e recomendações de prudência aplicam-se.

Atenção

H317	Pode provocar uma reacção alérgica cutânea.
P261	Evitar respirar os aerossóis.
P280	Usar luvas de protecção/ vestuário de protecção.
P302+P352	SE ENTRAR EM CONTACTO COM A PELE: lavar abundantemente com sabão água.
P333+P313	Em caso de irritação ou erupção cutânea: consulte um médico.
P362+P364	Retirar a roupa contaminada e lavá-la antes de a voltar a usar.

Mais informações podem ser encontradas na ficha de dados de segurança.

12.2. Considerações de Eliminação

Resíduos de químicos e preparações são geralmente considerados como resíduos perigosos. A eliminação deste tipo de resíduos está regulada por leis e normativas nacionais e regionais. Contactar as autoridades locais ou empresas de gestão de resíduos as quais podem aconselhar sobre como eliminar resíduos perigosos.

13. INFORMAÇÃO DE PEDIDO

Prod. No.:	MYCM0350	Mycoplasma pneumoniae IgM ELISA (96 Determinações)

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ABBREVIATIONS / ABKÜRZUNGEN / ABRÉVIATIONS / ABBREVIAZIONI / ABREVIACIÓNES / ABREVIATURAS

CMIT	5-chloro-2-methyl-4-isothiazolin-3-one
MIT	2-methyl-2H-isothiazol-3-one

SYMBOLS KEY / SYMBOLSCHLÜSSEL / EXPLICATION DES SYMBOLES / LEGENDA / SIMBOLOS / TABELA DE SIMBOLOS

	Manufactured by / Hergestellt von / Fabriqué par / Prodotto da / Fabricado por / Fabricado por
IVD	In Vitro Diagnostic Medical Device / In Vitro Diagnosticum / Dispositif médical de diagnostic in vitro / Diagnostico in vitro / Producto para diagnóstico In vitro / Dispositivo Médico para Diagnóstico In Vitro
LOT	Lot Number / Chargenbezeichnung / Numéro de lot / Lotto / Número de lote / Número de lote
	Expiration Date / Verfallsdatum / Date de péremption / Scadenza / Fecha de caducidad / Data de Validade
	Storage Temperature / Lagertemperatur / Température de conservation / Temperatura di conservazione / Temperatura de almacenamiento / Temperatura de Armazenamento
CE	CE Mark / CE-Zeichen / Marquage CE / Marchio CE / Marca CE / Marca CE
REF	Catalogue Number / Katalog Nummer / Référence du catalogue / Numero di codice / Número de Catálogo / Número de Catálogo
	Consult Instructions for Use / Arbeitsanleitung beachten / Consulter la notice d'utilisation / Consultare le istruzioni per l'uso/ Consulte las Instrucciones de Uso / Consultar as Instruções de Utilização
MTP	Microtiterplate / Mikrotiterplatte / Plaque de Microtitrage / Piastre di Microtitolazione / Placa de Microtitulação / Placa de Microtitulação
CONJ	Conjugate / Konjugat / Conjugué / Coniugato / Conjugado / Conjugado
CONTROL | -	Negative Control / Negativkontrolle / Contrôle Négatif / Controllo Negativo / Control Negativo / Controle Negativo
CONTROL | +	Positive Control / Positivkontrolle / Contrôle Positif / Controllo Positivo / Control Positivo / Controle Positivo
CUT OFF	Cut-off Control / Cut-off Kontrolle / Contrôle Cut-off / Controllo Cut-off / Control Cut-off / Controle Cut-off
DIL | M	IgM Sample Dilution Buffer / IgM-Probenverdünnungspuffer / Tampon de Dilution d'Échantillon IgM / Tampone di Diluizione del Campione IgM / Tampón de Dilución de Muestras IgM/ Tampão de Diluição de Amostra IgM
SOLN | STOP	Stop Solution / Stopplösung / Solution d'Arrêt / Soluzione Bloccante / Solución de Parada/ Solução de Bloqueio
SUB | TMB	TMB Substrate solution / TMB-Substratlösung / Solution de Substrat TMB / Soluzione Substrato TMB / Solución de Sustrato de TMB / Solução Substrato TMB
WASH | BUF | 20x	Washing Buffer 20x concentrated / Waschpuffer 20x konzentriert / Tampon de Lavage concentré 20 x / Tampone di Lavaggio concentrazione x20 / Tampón de Lavado concentrado x20 / Tampão de Lavagem concentrada 20x
	Contains sufficient for "n" tests / Ausreichend für "n" Tests / Contenu suffisant pour "n" tests / Contenuto sufficiente per "n" saggi / Contenido suficiente para "n" tests / Conteúdo suficiente para "n" testes

SUMMARY OF TEST PROCEDURE / KURZANLEITUNG TESTDURCHFÜHRUNG / RÉSUMÉ DE LA PROCEDURE DE TEST / SCHEMA DELLA PROCEDURA / RESUMEN DE LA TÉCNICA / RESUMO DO PROCEDIMENTO DE TESTE

SCHEME OF THE ASSAY

Mycoplasma pneumoniae IgM ELISA

Test Preparation

Prepare reagents and samples as described. Establish the distribution and identification plan for all samples and standards/controls on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Assay Procedure

	Substrate Blank (A1)	Negative Control	Cut-off Control	Positive Control	Sample (diluted 1+100)
Negative Control	-	100 µL	-	-	-
Cut-off Control	-	-	100 µL	-	-
Positive Control	-	-	-	100 µL	-
Sample (diluted 1+100)	-	-	-	-	100 µL
Cover wells with foil supplied in the kit **Incubate for 1 h at 37 ± 1 °C** Wash each well three times with 300 µL of Washing Buffer					
Conjugate	-	100 µL	100 µL	100 µL	100 µL
Incubate for 30 min at room temperature (20...25 °C) Do not expose to direct sunlight Wash each well three times with 300 µL of Washing Buffer					
TMB Substrate solution	100 µL	100 µL	100 µL	100 µL	100 µL
Incubate for exactly 15 min at room temperature (20...25 °C) in the dark					
Stop Solution	100 µL	100 µL	100 µL	100 µL	100 µL
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

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Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Parathyroid Hormone, Intact (PTH) 2nd Generation Test System

Product Code: 9025-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Intact PTH Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Parathyroid hormone (PTH) is a polypeptide composed of 84-amino acids and vital to calcium homeostasis[1] regulating blood serum calcium (Ca^{2+}) in concert with Vitamin D and Calcitonin. Secreted by the parathyroid gland in response to low Ca^{2+}, PTH stimulates calcium release in the bone marrow, production in the intestines and kidney[2] and minimizes urinary excretion. Meanwhile calcitonin has the opposing effect to increase urinary excretion and reduce blood calcium when Ca^{2+} is at elevated levels[3].

Intact PTH clears quickly from the bloodstream with half-life of less than four minutes. Detecting elevated PTH levels is imperative in monitoring bone metabolism especially in the presence of hypercalcemia[4], which virtually makes the primary diagnosis of hyperparathyroidism, as the vast majority (>90%) of such patients have elevated PTH. Differentiation from other forms of (non-parathyroid-mediated) hypercalcemia such as malignancy (the second most common cause), sarcoidosis, and thyroid toxicosis are associated with suppressed levels of parathyroid hormone or PTH in normal range. In cases of hypocalcemia, PTH levels may not be detectable due to total hypoparathyroidism but are found in normal range in hypocalcemia due to partial loss or inhibition of parathyroid function. Clinical significance of parathyroid hormone has increased in conjunction with the etiology of hypocalcemia and hypercalcemia. Initial studies revealed parathyroid hormone is synthesized as a prohormone followed by significant cleavage and modification, with these fragments comprising the majority of circulating parathyroid hormone. However, PTH fragments lack biological activity, and intact PTH (IPTH) spanning residues 1-84 is responsible for calcium regulation. The N-terminus of PTH is necessary in receptor docking, while the C-terminal residues are responsible for PTH receptor activation[5,6]. Thus, separation of whole parathyroid hormone from fragmented peptides is integral in osteometabolic analysis[7].

3.0 PRINCIPLE

Sandwich Equilibrium Method (TYPE 2):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of x-PTH antibody (C terminal epitope) coated on the well.

Upon mixing the enzyme-labeled antibody (N-terminal epitope) and a serum containing the native antigen, a reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab + Ag_{PTH} + Ab_{(well)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{PTH} - Ab_{(well)}$$

$Ab_{(well)}$ = Antibody coated on well (Excess Quantity)
Ag_{PTH} = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{PTH} - Ab_{(well)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

After sufficient time results, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. PTH Calibrators – 1.0 ml/vial (Lyophilized) Icons [A – F]
Six (6) vials of references for PTH at levels of 0(**A**), 15(**B**), 75(**C**), 150(**D**), 500(**E**) and 1000(**F**) pg/ml. Store at 2-8 °C. ***Reconstitute each vial with 1.0ml of distilled or deionized water.*** The reconstituted calibrators are stable for 24 hours at 2-8 °C. To store for a longer period, aliquot the reconstituted calibrators into cryo vials and store at -20 °C. ***DO NOT FREEZE/ THAW MORE THAN TWICE.*** A preservative has been added.
Note: The calibrators, human serum based, are traceable to the WHO 1st IS standard 95/646.

B. PTH Controls – 1.0 ml/vial (Lyophilized) Icons [M&N]
Two (2) vials of reference controls for PTH. Store at 2-8 °C. ***Reconstitute each vial with 1.0ml of distilled or deionized water.*** The reconstituted controls are stable for 24 hours at 2-8°C. To store for a longer period, aliquot the reconstituted controls into cryo vials and store at -20°C. ***DO NOT FREEZE/ THAW MORE THAN TWICE.*** A preservative has been added.

C. PTH Enzyme Reagent 2nd Gen – 6ml/vial – Icon (E)
One (1) vial contains anti-PTH conjugate reagent. Store at 2-8 °C.

D. PTH Antibody Coated Plate – 96 wells – Icon
One 96-well microplate coated with x-PTH antibody. Store at 2-8 °C.

E. Wash Solution Concentrate (20x) – 20 ml/vial – Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8 °C. *See Reagent Preparation section.*

F. Substrate Reagent – 12 ml/vial – Icon S^N
One (1) vial contains tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8 °C.

G. Stop Solution – 8 ml/vial – Icon (STOP)
One (1) vial contains a strong acid (H_2SO_4). Store at 2-8 °C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Do not expose reagents to heat, sun, or strong light.
Note 3: The above components are for one 96-well microplate. For other kit configurations, please refer to the table at end of the insert.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum or EDTA plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants for serum or EDTA containing tubes for plasma. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

If the specimen(s) cannot be assayed immediately after blood withdrawal, the sample(s) may be stored at temperatures of -20 °C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing (a maximum of two freeze/thaw cycles prior to use). When assayed in duplicate, 0.100 ml (100 µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

Note: Do not use reagents that are contaminated or have bacterial growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27 °C).
*****Test Procedure should be performed by a skilled individual or trained professional*****
1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8 °C.**
2. Pipette 0.050 ml (50 µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50 µl) of the PTH Enzyme Reagent 2nd gen to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds, cover and incubate for 60 minutes at room temperature.
5. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
6. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
7. Add 0.100 ml (100 µl) of TMB Substrate to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE PLATE AFTER SUBSTRATE ADDITION
8. Incubate at room temperature for twenty (20) minutes.
9. Add 0.050 ml (50 µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
10. Read the absorbance in each well at 450nm (using a reference wavelength of 630nm to minimize well imperfections) in a microplate reader. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: For re-assaying specimens with concentrations greater than 1000 pg/ml, dilution should be performed in human serum or plasma with low PTH values and multiplied accordingly.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of PTH in unknown specimens.
1. Plot the absorbance for each duplicate serum reference versus the corresponding PTH concentration in pg/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
2. Draw the best-fit curve through the plotted points.
3. To determine the concentration of PTH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.800) intersects the dose response curve at 419 pg/ml PTH concentration (See Figure 1).

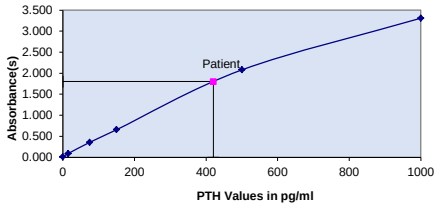
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (pg/ml)
Cal A	A1	0.013	0.015	0
	B1	0.017		
Cal B	C1	0.082	0.091	15
	D1	0.106		
Cal C	E1	0.370	0.357	75
	F1	0.355		
Cal D	G1	0.677	0.657	150
	H1	0.647		
Cal E	A2	2.103	2.079	500
	B2	2.065		
Cal F	C2	3.265	3.308	1000
	D2	3.360		
Patient	E2	1.801	1.800	**419**
	F2	1.800		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

FIGURE 1



*If the absorbance readout is off-scale or higher than the average absorbance of the highest calibrator, sample should be repeated with dilution.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. Maximum Absorbance (Calibrator 'F') ≥1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. pipettes, readers, washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. The reagents for AccuBind® ELISA procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. *(Boscato LM Stuart MC.'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem 1988:3427-33).* For diagnostic purposes the results from this assay should be used in combination with clinical examination, patient's history, and, all other clinical findings.

13.0 EXPECTED RANGES OF VALUES

Intact PTH levels were measured in fifty-eight (58) apparently normal individuals. The values obtained ranged from **9.0 to 94 pg/ml**. Based on the statistical tests for skewness and kurtosis, the data population when transformed logarithmically, follows the normal or Gaussian distribution as shown in histograms. The range of the geometric mean ± 2 standard deviations of the mean was calculated at **10.4 to 66.5 pg/ml**.

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within assay precision of the PTH AccuBind® Microplate EIA Test System were determined by analyses on six different levels of pool control and patient sera. The mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 1.

TABLE 1
Precision data for the PTH Test System

Sample	Mean Value (pg/ml)	Within-Run Precision		Total Precision (n=80)	
		SD	CV%	SD	CV%
Control 1	20.7	1.29	6.23	5.93	8.96
Control 2	66.1	2.88	4.35	8.47	4.65
Control 3	182.2	4	2.2	21.47	4.89
Patient 1	438.7	12.44	2.84	26.42	4.39
Patient 2	601.9	15.1	2.51	38.54	5.81
Patient 3	663.7	19.72	2.97	5.93	8.96

*As measured in forty experiments in duplicate over a 20 day period.

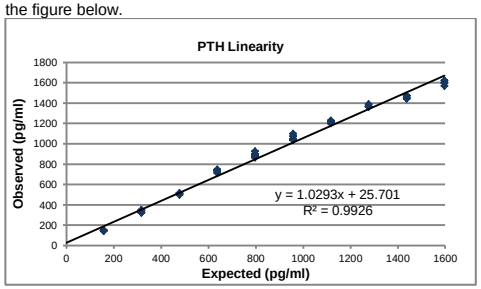
14.2 Sensitivity

The Intact PTH Accubind® ELISA test system has a LoB=1.393 pg/ml and a LoD=LoQ=1.501 pg/ml.

14.3 Accuracy

14.3.1 Linearity

The linearity of the Intact PTH Accubind® ELISA test system was tested by diluting human serum samples containing high levels of PTH (494-1597 pg/ml) with the "0 pg/ml" serum reference. The system produced excellent linearity up to 1597 pg/ml as shown in the figure below.



14.3.2 Recovery

The recovery of the Intact PTH AccuBind® Microplate ELISA Test System was calculated for five patient samples spiked with 50, 150, 250, 550, and 750 pg/ml PTH. Recoveries were determined to be within 15% of the expected values for all samples.

14.4 Specificity

The following fragments of PTH were tested and found to be non-reactive.

Peptide	Conc (pg/ml)	% Reactivity
1-34 fragment	100,000	0.001
1-44 fragment	100,000	0.005
7-34 fragment	100,000	0.002

14.5 High Dose Hook Effect:

The "high dose hook effect" of the PTH AccuBind® ELISA was assessed using several samples containing massive concentrations of PTH (10,000 -75,000 pg/ml).
The test showed no hook effect up to concentrations of 75,000 pg/ml. Samples with PTH concentrations greater than 75,000 pg/ml were not tested and may demonstrate hook effect. All samples that are greater than the highest calibrator should be diluted and measured again, however.

15.0 REFERENCES

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Effective Date: 2022-Mar-30 Rev. 4 **DCO: 1543**
MP9025 **Product Code: 902*5-300***

Size		96(A)	192(B)
Reagent (fill)	A)	1.0ml set	1.0ml set
	B)	1.0ml set	1.0ml set
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (12ml)	2 (12ml)
	G)	1 (8ml)	2 (8ml)

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13 3951 DBMaarn, The Neatherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device	2°-8° Temperature Limitation Storage Condition (2-8°C)	Consult Instructions for Use
REF Catalogue Number	Σ Contains Sufficient Test for Σ	LOT Batch Code
Used By (Expiration Day)	Date of Manufacturer	Manufacturer
EC REP Authorized Rep in European Country	CE European Conformity	

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Progesterone Test System
Product Code: 4825-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Progesterone Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of progesterone in serum or plasma is considered to be the most reliable way to assess its rate of production.

Progesterone is a steroid hormone, which plays an important role in the preparation for and maintenance of pregnancy. It is synthesized from cholesterol via pregnenolone — then rapidly metabolized to pregnanediol primarily in the liver.[2, 9,13] The ovary and placenta are the major production sites, but a small amount is also produced by the adrenal cortex in both men and women. Circulating progesterone levels, which are characteristically low during the follicular phase, increase sharply during the luteal phase of menstrual cycles, reaching a maximum approximately 5 to 10 days after the midcycle LH peak.[12] Unless pregnancy occurs, a steep decline to follicular levels sets in about 4 days before the next menstrual period. This pattern constitutes the rationale behind the well established use of serum progesterone measurements as a simple and reliable method for ovulation detection.[3,4,16]

For routine measurements, immunoassays using steroid specific antibodies are preferred. Initial immunoassays for serum progesterone used organic solvents to remove the steroid from endogenous binding proteins such as corticosteroid binding globulin (CBG) and albumin. Direct measurement of progesterone in serum or plasma is considered to be the method of choice for routine applications. Both RIA and EIA (and some FIA) are available in the market. Since RIA involves handling radioactivity and causes radioactive waste disposal issues, various non-isotopic methods have replaced the RIA. These methods use very specific antibodies to determine levels of progesterone in circulation.

The Monobind Progesterone ELISA kit uses a specific anti-progesterone antibody, and does not require sample extraction of serum or plasma. Cross-reactivity to other naturally occurring and structurally related steroids is low.

The employment of several serum references of known progesterone concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with progesterone concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the followed equation:

$$^{Enz}Ag + Ag + Ab_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

Ab_{Btn} = Biotinylated Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{Btn}$ = Antigen-Antibody Complex
$^{Enz}AgAb_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.
$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{CW} \Rightarrow \underline{immobilized\ complex}$
$\underline{Streptavidin}_{CW}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Progesterone Calibrators – 1ml/vial - Icons A-G
Seven (7) vials of serum reference for progesterone at concentrations of 0 (**A**), 0.3 (**B**), 2.0 (**C**), 5.0 (**D**), 15 (**E**), 30 (**F**) and 60.0 (**G**) ng/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nM/L) by multiplying by 3.18.
For example: 1ng/ml x 3.18 = 3.18 nM/L

B. Progesterone Enzyme Reagent – 6ml/vial – Icon
One (1) vial of Progesterone (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with red dye. Store at 2-8°C.

C. Progesterone Biotin Reagent – 6ml/vial - Icon ∇
One (1) vial of reagent contains anti-Progesterone biotinylated purified rabbit IgG conjugate in buffer, yellow dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells –Icon ⇓
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial – Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate Reagent – 12ml/vial - Icon S^N
One (1) vial contains tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon
One (1) vial contains a strong acid (H_2SO_4). Store at 2-8°C.

H. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on label.**
Note 3: Above reagents are for a single 96-well Microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025 & 0.050ml (25 & 50µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or heparanised plasma in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube or for plasma use evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8oC for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20oC for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20 -27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control or patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050ml (50µl) of Progesterone Enzyme Reagent to all wells.
4. Swirl the microplate gently for 10-20 seconds to mix.
5. Add 0.050ml (50µl) Progesterone Biotin Reagent to all wells.
6. Swirl the microplate gently for 10-20 seconds to mix.
7. Cover and incubate for 60 minutes at room temperature.
8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
10. Add 0.100ml (100µl) of Substrate reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

11. Incubate at room temperature for twenty (20) minutes.
12. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 60ng/ml 1:5 and 1:10 with progesterone '0' ng/ml calibrator or male patient serum pools with a known low value for progesterone.

10.0 CALCULATION OF RESULTS

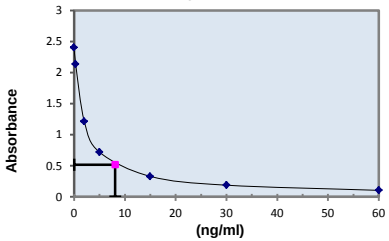
A dose response curve is used to ascertain the concentration of progesterone in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding progesterone concentration in ng/ml on linear graph paper.
3. Connect the points with a best-fit curve.
4. To determine the concentration of progesterone for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.517) intersects the dose response curve at 8.1ng/ml progesterone concentration.

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.420	2.406	0
	B1	2.391		
Cal B	C1	2.155	2.137	0.3
	D1	2.119		
Cal C	E1	1.248	1.215	2.0
	F1	1.183		
Cal D	G1	0.721	0.719	5.0
	H1	0.717		
Cal E	A2	0.338	0.330	15.0
	B2	0.322		
Cal F	C2	0.187	0.188	30.0
	D2	0.190		
Cal G	G2	0.107	0.105	60.0
	H2	0.104		
Pat# 1	A3	0.525	0.517	8.1
	B3	0.510		

Figure 1



*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 ng/ml should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available upon request from Monobind, Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. Patient specimens with Progesterone levels higher than 60ng/ml may be diluted (1:5 or 1:10) with progesterone '0 ng/ml' calibrator or male patient serum pools with a known low value for progesterone.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedures have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. "Heterophilic antibodies: a problem for all immunoassays" *Clin.Chem.* 1988:3427-33). For diagnostic purposes, results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population and females during gestation the expected ranges for the Progesterone AccuBind® ELISA Test System are detailed in Table 1. During pregnancy the progesterone serum levels rise rapidly till the end of third trimester.[17]

TABLE I
Expected Values for the Progesterone Test System

	(ng/ml)	(nmol/L)
Prepubertal Child (1-10 yr)	0.07 – 0.52	0.2-1.7
Adult man	0.13 – 1.22	0.4 – 3.88
Adult woman		
Follicular phase	0.15 – 1.40	0.5 – 4.4
Luteal phase	2.0 – 25.0	6.4 – 79.5
Pregnant woman		
First trimester	7.25 – 90.0	23 – 286
Second trimester	19.5 – 91.0	62 – 289
Third trimester	49.0 – 422.0	153 – 1342
Postmenopausal woman	0.0 – 0.80	0.0 – 2.55

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer until an in-house range can be determined by analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Progesterone AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.%
Low	20	0.65	0.100	15.3
Normal	20	10.77	0.405	3.8
High	20	24.94	1.528	6.1

TABLE 3
Between Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.%
Low	20	0.72	0.065	8.9
Normal	20	10.88	0.846	7.5
High	20	24.05	1.534	6.4

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The Progesterone AccuBind® ELISA Test System has a sensitivity of 0.105 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Progesterone AccuBind® ELISA Test System was compared with a chemiluminescence immunoassay method. Biological specimens from low, normal and high progesterone level populations were used (values ranged from < 0.15 ng/ml – 128 ng/ml). The total number of such specimens was 60. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method (y)	14.59	y= -1.223+1.018(x)	0.989
Reference (X)	15.53		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The % cross reactivity of the progesterone antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of progesterone needed to displace the same amount of labeled analog.

Substance	Cross Reactivity
Progesterone	100.000
17OH-Progesterone	0.375
Androstenedione	0.158
Cortisone	0.014
Corticosterone	0.347
Cortisol	0.005
Danazol	0.003
Dihydotestosterone	0.006
DHEA sulfate	0.002
Estradiol	0.004
Estrone	0.003
Estriol	0.002
Prednisone	0.023
Testosterone	0.015

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Revision: 7 Date: 2019-Jul-16 DCO: 1353
MP4825 Product Code: 4825-300

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (6ml)	2 (6ml)
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (12ml)	2 (12ml)
	G)	1 (8ml)	2 (8ml)

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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
Temperature Limitation Storage Condition (2-8°C)
Consult Instructions for Use
REF — Catalogue Number
Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Prolactin Hormone (PRL) Sequential Test System

Product Code: 4425-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Prolactin Hormone Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Prolactin hormone (PRL), secreted from the lactotrophs of the anterior pituitary, is a protein consisting of a single polypeptide chain containing approximately 200 amino acids. The primary biological action of the hormone is on the mammary gland where it is involved in the growth of the gland and in the induction and maintenance of milk production. There is evidence to suggest that prolactin may be involved in steroidogenesis in the gonad, acting synergistically with luteinizing hormone (LH). High levels of prolactin appear to inhibit steroidogenesis as well as inhibiting LH and follicle stimulating hormone (FSH) synthesis at the pituitary gland.[1,2]

The clinical usefulness of the measurement of prolactin hormone (PRL) in ascertaining the diagnosis of hyperprolactinemia and for the subsequent monitoring the effectiveness of the treatment has been well established.[3,4]

In this method, PRL calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal antibody (specific for PRL) is added and the reactants mixed. Reaction between the PRL antibodies and native PRL forms complex that binds with the streptavidin coated to the well. The excess serum proteins are washed away via a wash step. An enzyme labeled monoclonal antibody specific to PRL (different epitope from the biotinylated antibody) is added to the wells. The enzyme labeled antibody binds to the PRL already immobilized on the well through its binding with the biotinylated monoclonal antibody. Excess enzyme is washed off via a wash step. A color is generated by the addition of a substrate. The intensity of the color generation is directly proportional to the concentration of the PRL in the sample.

3.0 PRINCIPLE

Immunoenzymometric sequential assay (TYPE 4):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-prolactin antibody.

Upon mixing monoclonal biotinylated antibody, and a serum containing the native antigen, reaction results between the native antigen and the antibody, forming an antibody-antigen complex. The interaction is illustrated by the following equation:

$$Ag_{(Prl)} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} Ag_{(Prl)} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
$Ag_{(Prl)}$ = Native Antigen (Variable Quantity)
$Ag_{(Prl)} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibody complex (Variable Quantity)
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$Ag_{(Prl)} - {}^{Btn}Ab_{(m)} + \underline{\text{Streptavidin}}_{CW} \Rightarrow \underline{\text{Immobilized complex}}$ (IC)
$\underline{\text{Streptavidin}}_{CW}$ = Streptavidin immobilized on well
$\underline{\text{Immobilized complex}}$ (IC) = Ag-Ab bound to the well

After a suitable incubation period, the antibody-antigen bound fraction is separated from unbound antigen by decantation or aspiration. Another antibody (directed at a different epitope) labeled with an enzyme is added. Another interaction occurs to form an enzyme labeled antibody-antigen-biotinylated-antibody complex on the surface of the wells. Excess enzyme is washed off via a wash step. A suitable substrate is added to produce color measurable with the use of a microplate spectrophotometer. The enzyme activity on the well is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

$$(IC) + {}^{Enz}Ab_{(x\text{-}Prl)} \underset{k_{-b}}{\overset{k_b}{\rightleftharpoons}} {}^{Enz}Ab_{(x\text{-}Prl)} - IC$$

$^{Enz}Ab_{(x\text{-}Prl)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(x\text{-}Prl)} - IC$ = Antigen-Antibodies Complex
k_b = Rate Constant of Association
k_{-b} = Rate Constant of Dissociation

4.0 REAGENTS

Materials Provided:

A. PRL-seq Calibrators –1 ml/vial - Icons A-F
Six (6) vials of references for PRL antigen in human serum at levels of 0(**A**), 10(**B**), 25(**C**), 50(**D**), 100(**E**) and 250(**F**) ng/ml*. Store at 2-8°C. A preservative has been added.
***Note**: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 3rd, IS (84/500).

B. PRLs Biotin Reagent – 13ml/vial - Icon ∇
One (1) vial containing biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. PRLs Enzyme Reagent – 13ml/vial - Icon E
One (1) vial containing enzyme (HRP) labeled antibody, in buffer, dye, and preservative. Store at 2-8°C.

D. Streptavidin Coated Microplate – 96 wells - Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20 ml - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

G. Substrate B – 7ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Stop Solution – 8ml/vial – Icon (STOP)
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

I. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

4.1 Required But Not Provided:

1. Pipette capable of delivering 0.025ml (25µl) and 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate reader with 450 & 620nm filters.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Store dulited buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution** – Stable for one year
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of Prolactin Biotin Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 30 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of Prolactin Enzyme Reagent solution to all wells.
   DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION
9. Incubate 30 minutes at room temperature.
10. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
11. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
12. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
    DO NOT SHAKE PLATE AFTER SUBSTRATE ADDITION
13. Incubate at room temperature for fifteen (15) minutes.
14. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds). **Always add reagents in the same order to minimize reaction time differences between wells**
15. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of prolactin hormone (PRL) in unknown specimens.

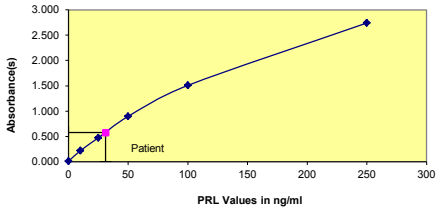
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding PRL concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of PRL for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.576) intersects the dose response curve at (31.1 ng/ml) PRL concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.012	0.010	0
	B1	0.007		
Cal B	C1	0.235	0.219	10
	D1	0.202		
Cal C	E1	0.482	0.474	25
	F1	0.465		
Cal D	G1	0.883	0.896	50
	H1	0.910		
Cal E	A2	1.537	1.508	100
	B2	1.480		
Cal F	C2	2.760	2.714	250
	D2	2.669		
Patient	G2	0.610	0.576	**31.1**
	H2	0.543		

Figure 1



The data presented in Example 1 and Figure 1 is for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

11.0 QC. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Patient specimens with abnormally high prolactin levels will not cause a hook effect, due to the assay design (sequential method). For specimens with values greater than 250, dilute the specimen 1/50 with '0' calibrator; and re-assay (multiply the result by 50).
8. Patients receiving preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA) and may show either falsely elevated or depressed values when assayed.
9. Pregnancy, lactation, and the administration of oral contraceptives can cause an increase in the level of Prolactin.
10. Drugs such as morphine, reserpine and the psychotropic drugs increase prolactin secretion.[5,6,7]
11. Since Prolactin hormone concentration is dependent upon diverse factors other than pituitary homeostasis, the determination alone is not sufficient to assess clinical status.

13.0 EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the PRL-seq AccuBind® ELISA Test System. The expected values (95% confidence intervals) are presented in Table 1.

TABLE I
Expected Values for the PRL-seq AccuBind® ELISA (in ng/ml)

	Women
Adult (Number = 70)	1.2 -- 19.5
Postmenopausal (Number = 10)	1.5 -- 18.5
	Men
Adult (Number = 50)	1.8 -- 17.0

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the PRL-seq AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation (σ) and coefficient of variation for each of these control sera are presented in Table 3 and Table 4.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	24	10.6	0.35	3.3%
Level 2	24	28.6	0.84	3.0%
Level 3	24	77.5	1.93	2.5%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	10	11.5	0.19	1.7%
Level 2	10	27.8	0.50	1.8%
Level 3	10	78.5	2.32	3.0%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

This procedure has a sensitivity of 0.04 ng. This is equivalent to a sample containing 0.8 ng/ml PRL concentration. The sensitivity was ascertained by determining the variability of the '0 ng/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The PRL-seq AccuBind® ELISA test system was compared with a reference method. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 86. The least square regression equation and the correlation coefficient were computed for the PRL ELISA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	19.0	y = 1.63 + 1.01(x)	0.973
Reference	17.3		

Only slight amounts of bias between the PRL-seq AccuBind® ELISA test system and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the prolactin hormone method to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of prolactin hormone needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Prolactin Hormone (PRL)	1.0000	--
Luteinizing Hormone (LH)	< 0.0001	1000ng/ml
Follitropin (FSH)	< 0.0001	1000ng/ml
Chorionic gonadotropin (CG)	< 0.0001	1000ng/ml
Thyrotropin (TSH)	< 0.0001	1000ng/ml
Growth Hormone (GH)	< 0.0001	1000ng/ml

15.0 REFERENCES

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11. Pasini F, Bergamini CM, Malfaccini M, Cocilovo G, Linciano M, Jacobs M, Bagni B, "Multiple molecular forms of prolactin during pregnancy in women", *J Endocrinol* **,** 106, 81-86 (1985).

Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP4425 Product Code: *4425-300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 (13ml)	2 (13ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (7ml)	2 (7ml)
	H)	1 (8ml)	2 (8ml)

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13
3951 DBMaarn, The Neatherlands
www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device	2°-8° Temperature Limitation Storage Condition (2-8° C)	Consult Instructions for Use	
REF Catalogue Number	Σ Contains Sufficient Test for Σ	LOT Batch Code	
Used By (Expiration Day)	Date of Manufacturer	Manufacturer	
EC REP Authorized Rep in European Country	CE European Conformity		

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Free Prostate Specific Antigen (fPSA) Test System

Product Code: 2325-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Free Prostate Specific Antigen (fPSA) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Prostate Specific antigen (PSA) is a serine protease with chymotrypsin-like activity.[1,2] The protein is a single chain glycoprotein with a molecular weight of 28.4 kDA.[3] PSA derives its name from the observation that it is a normal antigen of the prostate, but is not found in any other normal or malignant tissue. PSA is released from the normal prostate and appears at low serum concentrations in healthy men. Studies with reverse transcription-PCR have shown that PSA also is expressed at a low concentration in peripheral blood cells and other tissues.[4] High serum concentrations can be detected in patients with advanced prostate cancer (PCA).[5] Therefore, PSA is applied as a tumor marker for the clinical management of PCA.[6] However, increased PSA concentrations in serum also occur in patients with benign prostate hyperplasia (BPH).[7] Hence the goal is to discriminate clearly between BPH and PCA in the clinical laboratory to spare the patient invasive diagnostic procedures, such as a prostate biopsy.

In human serum, PSA occurs in two forms: free PSA (fPSA) and complexed PSA. The major form is a complex of PSA and α_1-antichymotrypsin (ACT). The fraction of fPSA was shown to be substantially smaller in patients with untreated PCA than in patients with BPH. Therefore, combined measurements of fPSA and total PSA (tPSA) may lead to a better discrimination between BPH and PCA Some recent studies have already shown that the fPSA/tPSA ratio is helpful in the differential diagnosis of BPH and PCA.

PSA is found in benign, malignant and metastatic prostate cancer. Since prostate cancer is the second most prevalent form of male malignancy, the detection of elevated PSA levels plays an important role in the early diagnosis. Serum PSA levels have been found to be more useful than prostatic acid phosphatase (PAP) in the diagnosis and management of patients due to increased sensitivity.[4]

In this method, fPSA calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different free epitopes of fPSA) are added and the reactants mixed. Reaction between the various fPSA antibodies and native fPSA forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-fPSA antibody bound conjugate is separated from the unbound enzyme-fPSA conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known prostate specific antigen (fPSA) levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with fPSA concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PSA antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, a reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(P)} + Ag_{fPSA} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)} - Ag_{fPSA} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Antibody (Excess Quantity)
Ag_{fPSA} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(p)} - Ag_{fPSA} - {}^{Btn}Ab_{(m)}$ =Antigen-Antibodies Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(p)} - Ag_{fPSA} - {}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow$ Immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. fPSA Calibrators – 1ml/vial - Icons A-F
Six (6) vials of serum references free PSA antigen at levels of 0(**A**), 0.5(**B**), 1.0(**C**), 2.5(**D**), 5.0(**E**) and 10.0(**F**) ng/ml. A preservative has been added. Store at 2-8°C.

Note: The calibrators, protein based buffered matrix, were calibrated using a reference preparation, which was assayed against the WHO 1st International Standard 96/668.

B. fPSA Enzyme Reagent – 13 ml/vial - Icon
One (1) vial containing enzyme labeled antibody, biotinylated specific free PSA monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C. (see Reagent Preparation Section).

E. Substrate A – 7ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

Materials Required But Not Provided:
1. Pipette capable of delivering 0.50 & 0.100ml (50 & 100µl) volume with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store at room temperature (2-30°C) for up to 60 days.
2. **Working Substrate Solution – Stable for one year**
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of the fPSA Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature (20-27°C).
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SUBTRATE ADDITION
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of fPSA in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding fPSA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of fPSA for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.648) intersects the dose response curve at 2.28ng/ml fPSA concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such

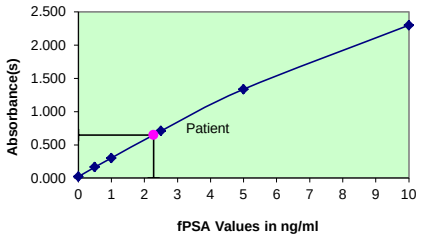
software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.019	0.021	0
	B1	0.022		
Cal B	C1	0.167	0.164	0.5
	D1	0.161		
Cal C	E1	0.300	0.302	1.0
	F1	0.304		
Cal D	G1	0.701	0.707	2.5
	H1	0.714		
Cal E	A2	1.353	1.337	5.0
	B2	1.321		
Cal F	C2	2.286	2.300	10.0
	D2	2.314		
Patient	E2	0.647	0.648	2.28
	F2	0.648		

*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with fPSA concentrations above 10 ng/ml may be diluted (for example 1/10 or higher) with normal female serum (PSA = 0 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed, is essential. Any deviation from Monobind IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis, as required by CE Mark IVD Directive 98/79/EC, for this and other devices made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (*Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33).* For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. fPSA is elevated in benign prostatic hyperplasia (BPH). Clinically an elevated fPSA value alone is not of diagnostic value as a specific test for differential diagnosis of BPH. The ratio of fPSA/tPSA is a better marker and should be used in conjunction with other clinical observations (DRE) and diagnostic procedures (prostate biopsy).
8. When the total PSA (tPSA) reads 4-10 ng/ml, the fPSA/tPSA ratio is useful in the differential diagnosis of BPH and PC (Prostate Cancer). Depending on the ratio, the probability can be determined as follows:

fPSA/tPSA Ratio	Probability of Prostate Cancer
0-10%	55%
10-15%	28%
15- 20%	25%
> 20%	10%

13.0 EXPECTED RANGE OF VALUES

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

TABLE I
Expected Values for the fPSA AccuBind® ELISA Test System

Healthy Males	≤ 1.3 ng/ml

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the fPSA AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	0.48	0.03	5.6%
Level 2	20	1.83	0.10	5.3%
Level 3	20	11.35	0.47	4.2%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	0.53	0.05	9.4%
Level 2	20	1.93	0.14	7.2%
Level 3	20	>11	-	-

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The theoretical sensitivity, or minimum detection limit, calculated by the interpolation of the mean plus two standard deviations of 20 replicates of the 0 ng/ml fPSA calibrator, is 0.008 ng/ml.

14.3 Accuracy

The fPSA AccuBind® ELISA test system was compared with a reference method. Clinical and non-clinical biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 167. The least square regression equation and the correlation coefficient were computed for the fPSA AccuBind® ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
Monobind (x)	1.62	x = 0.0189 + 0.9649(y)	0.957
Reference (y)	1.66		

Only slight amounts of bias between the fPSA AccuBind® ELISA test system and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity:

The following substances did not interfere with the performance of fPSA determination using the fPSA AccuBind® ELISA test system. These substances were added to the pooled sera in concentrations 10-100 times more than normal.

Compound	*Concentration Added*
AFP	10 µg/ml
Atropine	100 µg/ml
Acetylsalicylic Acid	100 µg/ml
Ascorbic Acid	100 µg/ml
Caffeine	100 µg/ml
Dexamethasone	10 µg/ml
Flutamide	100 µg/ml
hCG	100 IU/ml
hLH	100 IU/ml
Methotrexate	100 µg/ml
Prolactin	100 µg/ml
TSH	100 mIU/ml

15.0 REFERENCES

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Effective Date: 2019-Jul-16 Rev 5 **DCO: 1353**
MP2325 **Product Code: 2325-300**

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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IVD 2° 8° CE 0050

EC REP **CEpartner4U,** Esdoornlaan 13 3951 DBMaarn, The Neatherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8°C)
Consult Instructions for Use
REF — Catalogue Number
Σ — Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE 0050 — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Total Prostate Specific Antigen (PSA) Test System

Product Code: 2125-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Prostate Specific Antigen (PSA) Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Prostate Specific Antigen (PSA) is a serine protease with chymotrypsin-like activity.[1,2] The protein is a single chain glycoprotein with a molecular weight of 28.4 kDa.[3] PSA derives its name from the observation that it is a normal antigen of the prostate, but is not found in any other normal or malignant tissue.

PSA is found in benign, malignant and metastatic prostate cancer. Since prostate cancer is the second most prevalent form of male malignancy, the detection of elevated PSA levels plays an important role in the early diagnosis. Serum PSA levels have been found to be more useful than prostatic acid phosphatase (PAP) in the diagnosis and management of patients due to increased sensitivity.[4]

In this method, PSA calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of PSA) are added and the reactants mixed. Reaction between the various PSA antibodies and native PSA forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-PSA antibody bound conjugate is separated from the unbound enzyme-PSA conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known total prostate specific antigen (PSA) levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with PSA concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PSA antibody.
Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(p)} + Ag_{PSA} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)}\text{-}Ag_{PSA}\text{-}{}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ =Biotinylated Antibody (Excess Quantity)
Ag_{PSA} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(p)}\text{-}Ag_{PSA}\text{-}{}^{Btn}Ab_{(m)}$ =Antigen-Antibodies Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(p)}\text{-}Ag_{PSA}\text{-}{}^{Btn}Ab_{(m)}$+Streptavidin$_{C.W.}$⇒Immobilized complex
Streptavidin$_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. PSA Calibrators – 1 ml/vial – Icons A-F
Six (6) vials of serum references PSA Antigen at levels of 0(**A**), 5(**B**), 10(**C**), 25(**D**), 50(**E**) and 100(**F**) ng/ml. A preservative has been added. Store at 2-8°C.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the 1st IS 96/670.

B. PSA Enzyme Reagent – 13 ml/vial – Icon E
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/vial – Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C. (see Reagent Preparation Section).

E. Substrate A – 7 ml/vial – Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7 ml/vial – Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. (see Reagent Preparation Section).

G. Stop Solution – 8 ml/vial – Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8ºC. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a 96-well microplate. For other kit configurations, refer to the table at the end of this insert.

4.1 Required But Not Provided:
1. Pipette(s) capable of delivering 0.025, 0.050 & 0.100 ml (25, 50, & 100 µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate covers for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050 ml (50 µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Substrate Solution –** Stable for one year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20 - 27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8ºC.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the PSA Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 30 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of PSA in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding PSA concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of PSA for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.142) intersects the dose response curve at (23.6 ng/ml) PSA concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

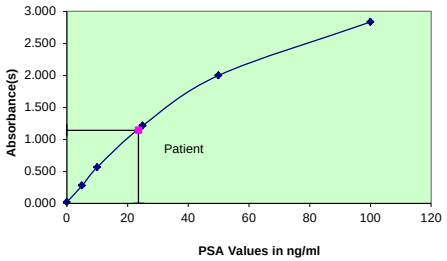
EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.019	0.019	0
	B1	0.019		
Cal B	C1	0.279	0.276	5
	D1	0.273		
Cal C	E1	0.567	0.563	10
	F1	0.559		
Cal D	G1	1.248	1.213	25
	H1	1.179		
Cal E	A2	2.051	1.999	50
	B2	1.947		
Cal F	C2	2.892	2.833	100
	D2	2.775		
Patient	E2	1.186	1.142	23.6

	F2	1.099		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with PSA concentrations above 100 ng/ml may be diluted (for example 1/10 or higher) with normal female serum (PSA = 0 ng/ml) and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor (10).
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis - as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for AccuBind® ELISA procedure have been formulated to eliminate maximal interference; however, potential interactions between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato, LM, Stuart, MC. "Heterophilic antibodies: a problem for all immunoassays" *Clin. Chem.* 1988: 3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. PSA is elevated in benign prostate hypertrophy (BPH). Clinically, an elevated **PSA value alone is not of diagnostic value as a specific test for cance**r and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures (prostate biopsy). Free PSA determinations may be helpful in regard to the discrimination of BPH and prostate cancer conditions.[5]
8. Due to the variation in the calibration used in PSA/ fPSA test kits and differences in epitopic recognition of different antibodies, it is always suggested that the patient sample should be tested with PSA/ fPSA tests made by the same manufacturer**. (*Monobind Inc. offers a fPSA ELISA test that should be used for consistency reasons, when needed.*)**

13.0 PERFORMANCE CHARACTERISTICS

Healthy males are expected to have values below 4 ng/ml.[4]

TABLE I
Expected Values for PSA AccuBind® ELISA Test System

Healthy Males	<4 ng/ml

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal"-persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the PSA AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	1.06	0.06	5.2%
Level 2	20	3.56	0.18	5.1%
Level 3	20	23.07	0.88	3.8%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	0.98	0.08	8.5%
Level 2	20	3.35	0.19	5.7%
Level 3	20	23.17	0.95	4.1%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The PSA AccuBind® ELISA test system has a sensitivity of 0.0003 ng/well. This is equivalent to a sample containing 0.013 ng/ml PSA concentration.

14.3 Accuracy

The PSA AccuBind® ELISA test system was compared with a reference Elisa method. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 241. The least square regression equation and the correlation coefficient were computed for the PSA AccuBind® ELISA test method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean	Least Square Regression Analysis	Correlation Coefficient
This Method (X)	5.62	y = -0.0598+0.98(X)	0.987
Reference (Y)	5.57		

Only slight amounts of bias between the PSA AccuBind® ELISA test system and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity:

No interference was detected with the performance of PSA AccuBind® ELISA test system upon addition of massive amounts of the following substances to a human serum pool.

Substance	Concentration
Acetylsalicylic Acid	100 µg/ml
Ascorbic Acid	100 µg/ml
Caffeine	100 µg/ml
CEA	10 µg/ml
AFP	10 µg/ml
CA-125	10,000 U/ml
hCG	1000 IU/ml
hLH	10 IU/ml
hTSH	100 mIU/ml
hPRL	100 µg/ml

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Revision: 6 Date: 2022-MAY-01 DCO: 1557
MP2125 Product Code: *2125-300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Σ — Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE 0050 — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Total Triiodothyronine (T3) Test System
Product Code: 125-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Triiodothyronine Concentration in T3 Calibrators or Plasma by a Microplate Enzyme Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of serum triiodothyronine concentration is generally regarded as a valuable tool in the diagnosis of thyroid dysfunction. This importance has provided the impetus for the significant improvement in assay methodology that has occurred in the last two decades. The advent of monospecific antiserum and the discovery of blocking agents to the T3 binding serum proteins have enabled the development of procedurally simple radioimmunoassays (1,2).
This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T3 conjugate is added, and then the reactants are mixed. A competition reaction results between the enzyme conjugate and the native triiodothyronine for a limited number of antibody combining sites immobilized on the well.
After the completion of the required incubation period, the antibody bound T3-enzyme conjugate is separated from the unbound T3-enzyme conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.
The employment of several serum references of known triiodothyronine concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with T3 concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 5):
The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.
Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubulized binding sites.

The interaction is illustrated by the following equation:

$$Enz_{Ag} + Ag + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{C.W.} + {}^{Enz}AgAb_{C.W.}$$

$Ab_{C.W.}$ = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
Enz_{Ag} = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{C.W.}$ = Antigen-Antibody Complex
$Enz_{Ag}Ab_{C.W.}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. T3 Calibrators References – 1ml/vial - Icons A-F
Six (6) vials of serum reference for triiodothyronine at concentrations of 0 (**A**), 0.5 (**B**), 1.0 (**C**), 2.5 (**D**), 5.0(**E**) and 7.5(**F**) ng/ml. Store at 2-8°C. A preservative has been added. **For SI units: ng/ml x 1.536 = nmol/L**

B. T3 Enzyme Reagent – 1.5ml/vial - Icon Ⓔ
One (1) vial of T3-horseradish peroxidase (HRP) conjugate in an albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C

C. T3/T4 Conjugate Buffer – 13ml - Icon Ⓑ
One (1) bottle reagent containing buffer, red dye, preservative, and binding protein inhibitors. Store at 2-8°C.

D. T3 Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with Sheep anti-T3 serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7 ml/vial - Icon S^A
One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

G. Substrate B – 7 ml/vial - Icon S^B
One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Stop Solution – 8ml/vial - Icon STOP
One (1) bottle of stop solution containing a strong acid (1N HCL). Store at 2-30°C.

I. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C. **Opened reagents are stable for sixty (60) days when stored at 2-8ºC. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a 96-well microplate. For other kit configurations, see table at end of IFU.

4.1 Materials Required But Not Provided:
1 Pipettes capable of delivering 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200µl) and (200-1000µl) dispenser(s) for conjugate and substrate preparation.
4. Microplate washers or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Test tubes for preparation of enzyme conjugate and substrate A plus B.
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate cover for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
11. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all T3 Calibrators products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay external controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. Working Reagent A - T3-enzyme Conjugate Solution
Dilute the T3-enzyme conjugate 1:11 with T3/T4 conjugate buffer in a suitable container. For example, dilute 160µl of conjugate with 1.6ml of buffer for 16 wells (A slight excess of solution is made). This reagent should be used within twenty-four hours for maximum performance of the assay. Store at 2-8°C.
General Formula:
Amount of Buffer required = Number of wells * 0.1
Quantity of T3-Enzyme necessary = # of wells * 0.01
i.e. = 16 x 0.1 = 1.6ml for Total T3/T4 Conjugate Buffer
16 x 0.01 = 0.16ml (160µl) for T3 enzyme conjugate

2. Wash Buffer
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.

3. Working Substrate Solution
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1 : Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8ºC.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of Working Reagent A, T3 Enzyme Reagent to all wells (see Reagent Preparation Section).
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: For re-assaying specimens with concentrations greater than 7.5ng/ml, pipette 25µl of the specimen and 25µl of the 0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the readout value by 2 to obtain the triiodothyronine concentration.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of triiodothyronine in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding T3 concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of T3 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis (y-axis) of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis (X-axis) of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.130) intersects the dose response curve at 1.95ng/ml T3 concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may be used for the data reduction. If such

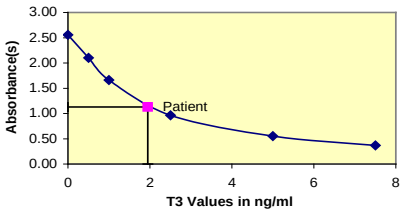
software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.604	2.556	0
	B1	2.507		
Cal B	C1	2.073	2.101	0.5
	D1	2.128		
Cal C	E1	1.678	1.662	1.0
	F1	1.646		
Cal D	G1	0.964	0.966	2.5
	H1	0.969		
Cal E	A2	0.550	0.551	5.0
	B2	0.551		
Cal F	C2	0.372	0.370	7.5
	D2	0.369		
Ctrl 1	E2	1.701	1.726	***0.92***
	F2	1.638		
Ctrl 2	G2	0.755	0.734	***3.58***
	H2	0.791		
Patient	A3	1.145	1.130	***1.95***
	B3	1.115		

*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 ng/ml should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with T3 concentrations above 7.5 ng/mL may be diluted ½ with '0' serum reference. The sample's concentration is obtained by multiplying the result by the dilution factor, 2.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. Total serum triiodothyronine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of triiodothyronine to TBG (3, 4). **Thus, total triiodothyronine concentration alone is not sufficient to assess clinical status.**
7. A decrease in total triiodothyronine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect total triiodothyronine values, has been compiled by the Journal of the American Association of Clinical Chemists[3].

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the T3 AccuBind™ ELISA Test System. The mean (R) values standard deviations (σ) and expected ranges (±2 σ) are presented in Table 1. The total number of samples was 105.

TABLE I
Expected Values for the T3 ELISA Test System (in ng/ml)

Mean (X)	**1.184**
Standard Deviation (σ)	0.334
Expected Ranges (±2 σ)	0.52 – 1.85

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the T3 AccuBind™ ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	16	0.78	0.06	7.9%
Normal	16	1.92	0.10	5.4%
High	16	3.55	0.14	3.9 %

TABLE 3
Between Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	10	0.76	0.07	8.9%
Normal	10	1.85	0.13	6.7%
High	10	3.43	0.16	4.5%

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The T3 AccuBind™ ELISA test system has a sensitivity of 0.04 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The T3 AccuBind™ ELISA method was compared with a reference radioimmunoassay method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.15ng/ml – 8.0ng/ml). The total number of such specimens was 120. The least square regression equation (y= mx+b) and the correlation coefficient were computed for the T3 AccuBind™ ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	1.62	y = 3.8 + 0.947(x)	0.987
Reference	1.68		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the triiodothyronine antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of triiodothyronine needed to displace the same amount of conjugate.

Substance	Cross Reactivity	Concentration
l-Triiodothyronine	1.0000	-
l-Thyroxine	< 0.0002	10µg/ml
Iodothyrosine	< 0.0001	10µg/ml
Diiodothyrosine	< 0.0001	10µg/ml
Diiodothyronine	< 0.0001	10µg/ml
Phenylbutazone	< 0.0001	10µg/ml
Sodium Salicylate	< 0.0001	10µg/ml

15.0 REFERENCES

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Revision: 4 **2022-May-01** **DCO: 1557**
Cat #: *125-300*

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (1.5ml)	2 (1.5ml)	1 (8ml)	2 (8ml)
	C)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	D)	1 plate	2 plates	5 plates	10 plates
	E)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	H)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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Monobind Inc.
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AccuBind
ELISA Microwells

Total Thyroxine (T4) Test System
Product Code: 225-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Thyroxine Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of serum thyroxine concentration is generally regarded as an important *in-vitro* diagnostic test for assessing thyroid function. This importance has provided the impetus for the significant improvement in assay methodology that has occurred in the last three decades. This procedural evolution can be traced from the empirical protein bound iodine (PBI) test (1) to the theoretically sophisticated radioimmunoassay (2).

This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T4 conjugate is added, and then the reactants are mixed. A competition reaction results between the enzyme conjugate and the native thyroxine for a limited number of antibody combining sites immobilized on the well.

After the completion of the required incubation period, the antibody bound enzyme-thyroxine conjugate is separated from the unbound enzyme-thyroxine conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known thyroxine concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with thyroxine concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 5)
The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.

Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubulized binding sites. The interaction is illustrated by the equation in the following below.

$$Enz_{Ag} + Ag + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{C.W.} + Enz_{AgAb_{C.W.}}$$

$Ab_{C.W}$ = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
Enz_{Ag} = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{C.W.}$ = Antigen-Antibody Complex
$Enz_{Ag\,Ab_{C.W.}}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

A. T4 **Calibrators – 1ml/vial - Icons A-F**
Six (6) vials of serum reference for thyroxine at concentrations of 0 (**A**), 2.0 (**B**), 5.0 (**C**), 10.0 (**D**), 15.0 (**E**) and 25.0 (**F**) µg/dl. Store at 2-8°C. A preservative has been added. **For SI units: µg/dl x 12.9 = nmol/L**

B. T4-Enzyme Reagent – 1.5ml/vial - Icon Ⓔ
One (1) vial of thyroxine-horseradish peroxidase (HRP) conjugate in a bovine albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C.

C. T3/T4 Conjugate Buffer – 13 ml - Icon Ⓑ
One (1) bottle reagent containing buffer, red dye, preservative, and binding protein inhibitors. Store at 2-8°C.

D. T4 Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with sheep anti-thyroxine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7ml/vial - Icon S^A
One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

G. Substrate B – 7ml/vial - Icon S^B
One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Stop Solution – 8ml/vial - Icon STOP
One (1) bottle containing a strong acid (1.0N HCl). Store at 2-8°C.

I. Product Insert.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8ºC. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a 96-well microplate. For other kit configurations, see table at the end of this IFU.

4.1 Required But Not Provided:
1. Pipette capable of delivering 25µl & 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200µl) and (200-1000µl) dispenser(s) for conjugate and substrate preparation
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Test tubes for preparation of enzyme conjugate.
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate cover for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
11. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PEPARATION

The specimens shall be blood; serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Working Reagent A = T4-Enzyme Conjugate Solution**
   Dilute the T4-enzyme conjugate 1:11 with Total T3/T4 conjugate buffer in a suitable container. For example, dilute 160µl of conjugate with 1.6ml of buffer for 16 wells (A slight excess of solution is made). This reagent should be used within twenty-four hours for maximum performance of the assay. Store at 2-8°C.
   General Formula:
   Amount of Buffer required = Number of wells * 0.1
   Quantity of T4 Enzyme necessary = # of wells * 0.01
   i.e. = 16 x 0.1 = 1.6ml for Total T3/T4 conjugate buffer
   16 x 0.01 = 0.16ml (160µl) for T4 enzyme conjugate
2. **Wash Buffer**
   Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
3. **Working Substrate Solution**
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1 : Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplate's wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8ºC.**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of Working Reagent A, T4 Enzyme Reagent to all wells (see Reagent Preparation Section).
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: For reassaying specimens with concentrations greater than 25 µg/dl, pipet 12.5µl of the specimen and 12.5µl of the 0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the readout value by 2 to obtain the thyroxine concentration.

10.0 CALCULATION OF RESULTS

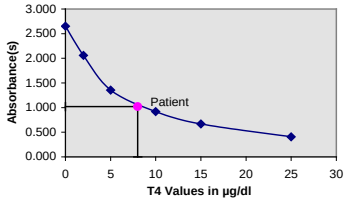
A dose response curve is used to ascertain the concentration of thyroxine in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding T4 concentration in µg/dl on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of T4 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in µg/dl) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.022) intersects the standard curve at (8 µg/dl) T4 concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (µg/dl)
Cal A	A1	2.648	2.650	0
	B1	2.652		
Cal B	C1	2.090	2.060	2
	D1	2.031		
Cal C	E1	1.344	1.355	5
	F1	1.366		
Cal D	G1	0.897	0.918	10
	H1	0.939		
Cal E	A2	0.676	0.668	15
	B2	0.659		
Cal F	C2	0.408	0.406	25
	D2	0.404		
Ctrl 1	E2	1.425	1.435	**4.6**
	F2	1.383		
Ctrl 2	G2	0.611	0.613	**16.3**
	H2	0.608		
Patient	A3	0.984	1.022	**8.0**
	B3	1.060		

EXAMPLE 1

Figure 1



The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 µg/dl should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with T4 concentrations greater than 35 µg/dl may be diluted ½ with the '0' serum reference into the sample well; pipet 12.5µl of the specimen and 12.5µl of the '0' serum reference in the sample well to maintain a uniform protein concentration. The sample's concentration is obtained by multiplying the result by the dilution factor, 2.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
2. Total serum thyroxine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of thyroxine to TBG (3, 4). **Thus, total thyroxine concentration alone is not sufficient to assess clinical status.**
3. Total serum thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives. A T3 uptake test may be performed to estimate the relative TBG concentration in order to determine if the elevated T4 is caused by TBG variation.
4. A decrease in total thyroxine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect total thyroxine values, has been compiled by the Journal of the American Association of Clinical Chemists.

"NOT INTENDED FOR NEWBORN SCREENING"

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the T4 AccuBind™ ELISA Test System. The mean (X) values, standard deviations (**σ**) and expected ranges (±2 **σ**) are presented in Table 1.

TABLE 1

Expected Values for the T4 ELISA Test System (in µg/dl)

	Male	Female *
Number of Specimens	42	58
Mean (X)	7.6	8.2
Std.Dev (σ)	1.6	1.7
Expected Ranges (±2 σ)	4.4 – 10.8	4.8 – 11.6

*Normal patients with high TBG levels were **not** excluded except if pregnant.

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the T4 AccuBind™ ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean values (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2

Within Assay Precision (Values in µg/dl)

Sample	N	X	σ	C.V.%
Low	20	6.87	0.16	2.3
Normal	20	9.95	0.16	1.6
High	20	13.13	0.17	1.3

TABLE 3

Between Assay Precision (Values in µg/dl)

Sample	N	X	σ	C.V.%
Low	20	5.76	0.37	6.3
Normal	20	9.41	0.57	6.1
High	20	16.18	1.21	7.5

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The T4 AccuBind™ ELISA test system has a sensitivity of 3.2ng/well. This is equivalent to a sample containing a concentration of 0.128 µg/dl. The sensitivity was ascertained by determining the variability of the 0 µg/dl serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The tT4 AccuBind™ ELISA method was compared with a coated tube radioimmunoassay method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.8µg/dl – 25µg/dl). The total number of such specimens was 131. The least square regression equation and the correlation coefficient were computed for the T4 AccuBind™ ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	8.07	y = 0.39+0.952(x)	0.934
Reference	8.06		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the thyroxine antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of thyroxine needed to displace the same amount of conjugate.

Substance	Cross Reactivity	Concentration
l–Thyroxine	1.0000	-
d-Thyroxine	0.9800	10µg/dl
d-Triiodothyronine	0.0150	100µg/dl
l–Triiodothyronine	0.0300	100µg/dl
Iodothyrosine	0.0001	100µg/ml
Diiodothyrosine	0.0001	100µg/ml
Diiodothyronine	0.0001	100µg/ml

15.0 REFERENCES

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Revision: 4 **Date: 2022-May-01** **DCO: 1557**

Cat #: *225-300*

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (1.5ml)	2 (1.5ml)	1 (8ml)	2 (8ml)
	C)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	D)	1 plate	2 plates	5 plates	10 plates
	E)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	H)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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Monobind Inc.
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AccuBind
ELISA Microwells

Free Triiodothyronine (Free T3) Test System

Product Code: 1325-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Free Triiodothyronine Concentration in Human Serum by a Microplate Enzyme Immunoassay. Levels of Free T3 are thought to reflect the amount of T3 available to the cells and may therefore determine the clinical metabolic status of T3.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Triiodothyronine, a thyroid hormone, circulates in blood bound to carrier proteins (1,2). The main transport protein is thyroxine-binding globulin (TBG). However, only the free (unbound) portion of triiodothyronine is believed to be responsible for the biological action. Further, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function as the concentrations of the carrier proteins alters, the total triiodothyronine level changes so that the free triiodothyronine concentration remains constant. Thus, measurements of free triiodothyronine concentrations correlate more reliably with clinical status than total triiodothyronine levels.

For example, the increase in total triiodothyronine levels associated with pregnancy, oral contraceptives and estrogen therapy result in higher total T3 levels while the free T3 concentration remains basically unchanged.

This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations in a direct determination of free T3. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T3 conjugate (analog method) is added, and then the reactants are mixed. A competition reaction results between the enzyme conjugate and the free triiodothyronine for a limited number of antibody combining sites immobilized on the well.

After the completion of the required incubation period, the antibody bound enzyme-triiodothyronine conjugate is separated from the unbound enzyme-triiodothyronine conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known free triiodothyronine concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with free triiodothyronine concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay TYPE 5
(Analog Method for Free T3)
The essential reagents required for a solid phase enzyme immunoassay include immobilized T3 antibody, enzyme-T3 conjugate and native free T3 antigen. The enzyme-T3 conjugate should have no measurable binding to serum proteins especially TBG and albumin. The method achieves this goal.

Upon mixing immobilized antibody, enzyme-T3 conjugate and a serum containing the native free T3 antigen, a competition reaction results between the native free T3 and the enzyme-T3 conjugate for a limited number of insolubulized binding sites. The interaction is illustrated by the following equation:

$$Enz_{Ag} + Ag + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{C.W.} + {}^{Enz}AgAb_{C.W.}$$

$Ab_{C.W.}$ = Monospecific Immobilized Antibody (Constant Quantity)

Ag = Native Antigen (Variable Quantity)

Enz$_{Ag}$ = Enzyme-antigen Conjugate (Constant Quantity)

$AgAb_{C.W.}$ = Antigen-Antibody Complex

Enz$_{Ag}$ $Ab_{C.W.}$ = Enzyme-antigen Conjugate -Antibody Complex

k_a = Rate Constant of Association

k_{-a} = Rate Constant of Disassociation

$K = k_a / k_{-a}$ = Equilibrium Constant

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided

A. Free T3 Calibrators – 1ml/vial - Icons A-F
Six (6) vials of serum reference for free triiodothyronine at **approximate*** concentrations of 0 (**A**), 1.0 (**B**), 3.0 (**C**), 5.0 (**D**), 8.0 (**E**) and 16.0 (**F**) pg/ml. Store at 2-8°C. A preservative has been added. For SI units use the conversion factor 1.536 to convert pg/ml to pmol/L.
* Exact levels are given on the labels on a lot specific basis.

B. Free T3- Enzyme Reagent – 13ml/vial - Icon
One (1) vial of triiodothyronine -horseradish peroxidase (HRP) conjugate in a bovine albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C.

C. T3 Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with sheep anti-triiodothyronine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20ml - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial - Icon S^A
One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7ml/vial - Icon S^B
One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon
One (1) bottle containing a strong acid (1N HCl). Store at 2-30°C.

H. Product Instructions.

4.1 Required But Not Provided:
1. Pipette capable of delivering 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a 96-well microplate. For other kit configurations, please see table at end of this IFU.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8^{0}C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20^{0}C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION:

1. **Wash Buffer**
   Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
2. **Working Substrate Solution**
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1 : Do not use the working substrate if it looks blue.

Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of fT3-Enzyme Reagent solution to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of free triiodothyronine in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding Free T3 concentration in pg/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of Free T3 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.855) (intersects the standard curve at (2.1pg/ml) Free T3 concentration (See Figure 1).

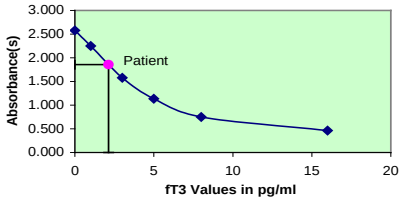
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value* (pg/ml)
Cal A	A1	2.658	2.579	0.0
	B1	2.531		
Cal B	C1	2.264	2.248	1.0
	D1	2,233		
Cal C	E1	1.570	1.578	3.0
	F1	1.585		
Cal D	G1	1.124	1.135	5.0
	H1	1.145		
Cal E	A2	0.749	0.748	8.0
	B2	0.748		
Cal F	C2	0.463	0.463	16.0
	D2	0.462		
Patient	E2	1.860	1.855	***2.1***
	F2	1.849		

The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a standard curve prepared with each assay**. Assigned values for calibrators are lot specific.**

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator A should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges

.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. If a patient, for some reason, reads higher than the highest calibrator report as such (e.g. > 16pg/ml). ***Do not try to dilute the sample. TBG variations in different matrices will not allow Free T3 hormone to dilute serially.***
7. Several drugs are known to affect the binding of Triiodothyronine to the thyroid hormone carrier proteins or its metabolism to T3 and complicate the interpretation of free T3 results (3).
8. Circulating autoantibodies to T3 and hormone-binding inhibitors may interfere (4).
9. Heparin has been reported to have *in vivo* and *in vitro* effects on free T3 concentration (5). Therefore, do not obtain samples in which this anti-coagulant has been used.
10. In severe nonthyroidal illness (NTI), the assessment of thyroid status becomes very difficult. TSH measurements are recommended to identify thyroid dysfunction (6).
11. Familial dysalbuminemic conditions may yield erroneous results on direct free T3 assays (7).

"NOT INTENDED FOR NEWBORN SCREENING"

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the fT3 AccuBind™ ELISA test system. The mean values (X), standard deviations (σ.) and expected ranges (±2σ.) are presented in Table 1.

TABLE I
Expected Values for Free T3 ELISA Test System (in pg/ml)

	Adult	Pregnancy
Number Specimens	110	75
Mean (X)	2.8	3.0
Standard Deviation (σ)	0.7	0.6
Expected Ranges (±2 σ)	1.4 – 4.2	1.8 – 4.2

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the Free T3 AccuBind™ ELISA test system were determined by analyses on three different levels of pool control sera. The number (N), mean values (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V.
Low	24	1.85	0.09	4.9%
Normal	24	4.49	0.16	3.6%
High	24	8.00	0.25	3.1%

TABLE 3
Between Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V.
Low	12	2.16	0.29	13.1%
Normal	12	5.09	0.40	7.9%
High	12	9.13	0.94	10.2%

*As measured in twelve experiments in duplicate.

14.2 Sensitivity

The FreeT3 AccuBind™ ELISA test system has a sensitivity of 0.410 pg/ml. The sensitivity was ascertained by determining the variability of the 0 pg/ml serum calibrator and using the 2 σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Free T3 AccuBind™ ELISA test system was compared with a coated tube radioimmunoassay analog method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.1pg/ml – 14pg/ml). The total number of such specimens was 151. The least square regression equation and the correlation coefficient were computed for this Free T3 AccuBind™ ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method (Y)	3.05	y = 0.35+0.922(x)	0.902
Reference (X)	2.92		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the triiodothyronine antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of triiodothyronine needed to displace the same amount of conjugate.

SUBSTANCE	Cross Reactivity	Concentration
L-Triiodothyronine	1.0000	----
L-Thyroxine	< 0.0002	10 µg/ml
Iodothyrosine	< 0.0001	10 µg/ml
Diiodothyrosine	< 0.0001	10 µg/ml
Diiodothyronine	< 0.0001	10 µg/ml
Phenylbutazone	< 0.0001	10 µg/ml
Sodium Salicylate	< 0.0001	10 µg/ml

15.0 REFERENCES

1. Pederson KO, *Scand J Clin Lab Invest,* **34,** 247 (1974).
2. Wild D, *Immunoassay Handbook*, Stockton Press**,** 339 (1994).
3. Wenzel KW, *Metabolism,* **30,** 717 (1981).
4. Bhagat C, et al, *Clin Chem,* **29,** 1324 (1983).
5. Lundberg PR, et al, *Clin Chem,* **28,** 1241 (1982).
6. Melmed S, et al, *J Clin Endocrinol Metab***, 54,** 300 (1982).
7. Lalloz MR et al, *Clin Endocrinol*, **18,** 11 (1983).

Revision: 5 **Date: 2022-May-01** **DCO: 1557**

Cat #: *1325-300*

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	C)	1 plate	2 plates	5 plates	10 plates
	D)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	E)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13
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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2°-8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Free Thyroxine (Free T4) Test System

Product Code: 1225-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Free Thyroxine Concentration in Human Serum by a Microplate Enzyme Immunoassay

2.0 SUMMARY AND EXPLANATION OF THE TEST

Thyroxine, the principal thyroid hormone, circulates in blood almost completely bound to carrier proteins. The main carrier is thyroxine-binding globulin (TBG). However, only the free (unbound) portion of thyroxine is responsible for the biological action. Further, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function as the concentrations of the carrier proteins alters, the total thyroxine level changes so that the free thyroxine concentration remains constant. Thus, measurements of free thyroxine concentrations correlate better with clinical status than total thyroxine levels.

The increase in total thyroxine associated with pregnancy, oral contraceptives and estrogen therapy occasionally result in total T4 levels over the limits of normal while the free thyroxine concentration remains in the normal reference range. Masking of abnormal thyroid function can also occur in both hyper and hypothyroid conditions by alterations in the TBG concentration. The total T4 can be elevated or lowered by TBG changes such that the normal reference levels result. The free thyroxine concentration can help in uncovering the patient's actual clinical status.

In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T4 conjugate (analog method) is added and the reactants are mixed. A competition reaction results between the enzyme conjugate and the free thyroxine for a limited number of antibody combining sites immobilized on the well.

After the completion of the required incubation period, the antibody bound enzyme-thyroxine conjugate is separated from the unbound enzyme-thyroxine conjugate via a wash step. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known free thyroxine concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with free thyroxine concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay, Analog Method for Free-T4 (TYPE 5):

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native free antigen, a competition reaction results between the native free antigen and the enzyme-antigen conjugate for a limited number of insolubilized binding sites. The interaction is illustrated by the followed equation:

$$Enz_{Ag} + Ag + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{C.W.} + {}^{Enz}AgAb_{C.W.}$$

$Ab_{C.W.}$ = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{C.W.}$ = Antigen-Antibody Complex
$^{Enz}Ag\,Ab_{C.W.}$ = Enzyme-antigen Conjugate -Antibody Complex
K_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native free antigen concentration. By utilizing several different serum references of known antigen con-centration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Free T4 Calibrators – 1 ml/vial - Icons A-F
Six (6) vials of human serum based reference calibrators for free thyroxine at **approximate*** concentrations of 0 (**A**), 0.40 (**B**), 1.25 (**C**), 2.10 (**D**), 5.00 (**E**) and 7.40 (**F**) ng/dl. Store at 2-8°C. A preservative has been added. For SI units use the conversion factor 12.9 to convert ng/dl to pmol/L.
* Exact levels are given on the labels on a lot specific basis.

B. fT4- Enzyme Reagent – 13 ml/vial - Icon
One (1) vial of thyroxine-horseradish peroxidase (HRP) conjugate in a protein-stabilized matrix. A preservative has been added. Store at 2-8°C.

C. Free T4 Antibody Coated Plate – 96 wells - Icon
One 96-well microplate coated with anti-thyroxine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20ml - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7 ml/vial - Icon S^A
One (1) bottle containing tetramethylbenzidine (TMB) in acetate buffer. Store at 2-8°C.

F. Substrate B – 7 ml/vial - Icon S^B
One (1) bottle containing hydrogen peroxide (H_2O_2) in acetate buffer. Store at 2-8°C.

G. Stop Solution – 8 ml/vial - Icon
One (1) bottle containing a strong acid (1N HCl). Store at 2-8 °C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a 96-well microplate. For other kit configurations, please refer to the table at the end of this IFU.

4.1 Materials Required But Not Provided:

1. Pipette capable of delivering 50µl & 100µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.
Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8^{0}C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20^{0}C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION:

1. **Wash Buffer**
   Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.
2. **Working Substrate Solution**
   Pour the contents of the plastic vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1 : Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of fT4 Enzyme Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

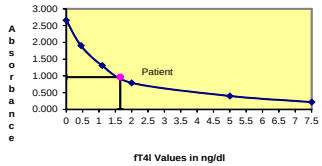
10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of free T4 in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding Free T4 concentration in ng/dl on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of Free T4 for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/dl) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.964) intersects the dose response curve at (1.65ng/dl) free T4 concentration (See Figure 1).

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a standard curve prepared with each assay. **Assigned values for calibrators are lot specific.**

Figure 1



EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value* (ng/dl)
Cal A	A1	2.658	2.612	0.00
	B1	2.566		
Cal B	C1	1.919	1.900	0.45
	D1	1.880		
Cal C	E1	1.339	1.306	1.10
	F1	1.273		

Cal D	G1	0.769	0.790	2.00
	H1	0.811		
Cal E	A2	0.396	0.400	5.00
	B2	0.404		
Cal F	C2	0.215	0.217	7.40
	D2	0.219		
Ctrl 1	E2	1.827	1.835	**0.50**
	F2	1.843		
Ctrl 2	G2	0.541	0.557	**2.70**
	H2	0.573		
Patient	A3	0.951	0.964	**1.65**
	B3	0.976		

Note 1: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 ng/dl should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. If a patient, for some reason, reads higher than the highest calibrator report as such (e.g. > 7.4 ng/dl). ***Do not try to dilute the sample. TBG variations in different matrices will not allow Free T4 hormone to dilute serially.***
7. Serum free-thyroxine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, Thyroxine binding globulin (TBG) concentration, and the binding of Thyroxine to TBG (3, 4). Thus, free-Thyroxine concentration alone is not sufficient to assess the clinical status.
8. Serum free-thyroxine values may be elevated under conditions such as pregnancy or administration of oral contraceptives.
9. A decrease in free thyroxine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect free Thyroxine values, has been compiled by the Journal of the American Association of Clinical Chemists.
10. The interpretation of Free T4 is complicated by a variety of drugs that can affect the binding of T4 to the thyroid hormone carrier proteins or interfere in its metabolism to T3. In severe non-thyroidal illness (NTI) the assessment of thyroid becomes especially difficult. Since the patients in this category may suffer from concomitant primary hypothyroidism or from compensatory secondary hypothyroidism. In cases like these a sensitive TSH evaluation of the patient may be recommended. Please see Monobind Cat# 325-300.
11. In rare conditions associated with extreme variations in albumin binding capacity for T4- such as familial dysalbuminemic hyperthyroxinemia (FDH) – direct assessment of Free T4 may be misleading.
12. Circulating antibodies to T4 and hormone binding inhibitors may interfere in the performance of the assay.
13. Heparin is reported to have in vivo and in vitro effects on free T4 levels. Samples from patients undergoing heparin therapy should be collected well before the administration of the anticoagulant.

"NOT INTENDED FOR NEWBORN SCREENING"

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the Free T4 AccuBind® ELISA test system. The mean (X) values, standard deviations (σ) and expected ranges (±2σ) are presented in Table 1.

TABLE 1
Expected Values for Free T4 ELISA Test System (in ng/dl)

	Adult	Pregnancy
Number of Specimens	89	31
Mean (X)	1.40	1.50
Standard Deviation (σ)	0.30	0.37
Expected Ranges (±2 σ)	0.8 – 2.0	0.76 – 2.24

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The *inter* and *intra* assay precisions of the Free T4 AccuBind® ELISA test system were determined by analyses on three different levels of pooled patient sera. The number (n), mean values (x), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

In order to validate the *intra-assay* precision of the Free T4 AccuBind® ELISA test system, twenty replicates of each of three pooled sera (low medium and high ranges of the dose response curve) were assayed in the same assay. An intra-assay precision of 3.25 to 10.98% was obtained.

TABLE 2
Intra-Assay Precision (in ng/dl)

Sample	N	X	σ	C.V.
Low	20	0.550	0.061	10.98%
Medium	20	1.740	0.074	4.26%
High	20	3.250	0.106	3.25%

In order to validate the *inter-assay* precision of fT4 AccuBind® ELISA test system, one duplicate of each of three pooled sera (low medium and high ranges of the dose response curve) was assayed in 10 assays done over a period of six months that involved five different sets of reagents and three different technicians. An inter-assay precision of 6.01 to 10.81% was obtained.

TABLE 3
Inter-Assay Precision (in ng/dl)

Sample	N	X	σ	C.V.
Low	10	0.480	0.052	10.81%
Medium	10	1.410	0.085	6.01%
High	10	3.490	0.279	7.90%

14.2 Sensitivity

The Free T4 AccuBind® ELISA test system has a sensitivity of 0.162 ng/dl. The sensitivity was ascertained by determining the variability of the 0 ng/dl serum calibrator and using the 2σ (95% certainty) statistics to calculate the minimum dose.

14.3 Accuracy

The Free T4 AccuBind® ELISA test system was compared with a coated tube radioimmunoassay (**RIA)** method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.1ng/dl – 8ng/dl). The total number of such specimens was 197. The least square regression equation and the correlation coefficient were computed for this Free T4 AccuBind® ELISA method in comparison with the predicate method (Table 4).

TABLE 4
Linear Regression Analysis

Method	Mean (x)	Equation	Correlation Coefficient
Monobind EIA "X"	1.56	y = 0.1034 + 0.9525x	0.920
Predicate RIA "Y"	1.59		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values.

14.4 Specificity:

The cross-reactivity of the thyroxine antibody used for Free T4 AccuBind® ELISA to selected substances was evaluated by adding massive amounts of the interfering substance to a serum matrix. The cross-reactivity was calculated by deriving a ratio between doses of interfering substance to dose of thyroxine needed to displace the same amount of the conjugate.

Substance	Cross Reactivity	Concentration
l–Thyroxine	1.0000	----
d-Thyroxine	0.9800	10µg/dl
d-Triiodothyronine	0.0150	100µg/dl
l–Triiodothyronine	0.0300	100µg/dl
Iodothyrosine	0.0001	100µg/ml
Diiodotyrosine	0.0001	100µg/ml
Diiodothyronine	0.0001	100µg/ml
TBG	N/D	40 µg/ml
Albumin	N/D	40 mg/ml
Phenylbutazone	N/D	10 µg/ml
Phenytoin	N/D	40 µg/ml
Salicylates	N/D	500 µg/ml

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Revision: 6 **Date: 2022-MAY-01** **DCO: 1557**
Cat #: *1225-300*

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	C)	1 plate	2 plates	5 plates	10 plates
	D)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	E)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (8ml)	2 (8ml)	1 (30ml)	2 (30ml)

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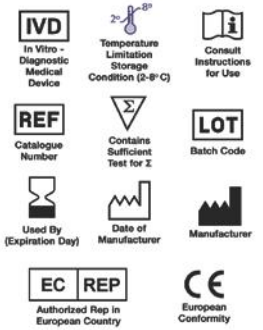
Glossary of Symbols
(EN 980/ISO 15223)



NovaTec Immundiagnostica GmbH

NovaLisa®

Taenia solium IgG

ELISA

CE

Only for in-vitro diagnostic use

Product Number: TAEG0420 (96 Determinations)

ENGLISH

1. INTRODUCTION

Taenia solium is a tapeworm of 2-7 m in length which resides in the small intestine of humans but also other animal species (monkeys, hamsters). The tapeworms produce proglottids (less than 1,000, and each with 50,000 eggs) which mature, become gravid, detach from the tapeworm, and migrate to the anus or are passed in the stool. The eggs contained in the gravid proglottids and passed with the faeces can survive for months to years in the environment. After ingestion of a suitable intermediate host (pigs and other animals, including humans) the eggs release the oncosphere, invade the intestinal wall and migrate to the striated muscles, into the brain, liver and other tissues of the host where they develop in cysticerci. In the human intestine, a cysticercus develops over 2 months into an adult tapeworm, which can survive for up to 25 years. The important parasitic infection caused by Taenia solium is cysticercosis which may involve the eye and the central nervous system. The swine tapeworm Taenia solium is worldwide in distribution. Prevalence is higher in poorer communities where humans live in close contact with pigs and eat undercooked pork, and is very rare in Muslim countries. The main symptom of Taeniasis (only mild) is often the passage (passive) of proglottids.

The most important feature of Taeniasis solium is the risk of development of Cysticercosis.

Species	Disease	Symptoms (e.g.)	Transmission route
Taenia solium	Taeniasis	Abdominal pain; Nausea; Weakness and fatigue; Weight loss; Flatulence (gases); Diarrhea or constipation; Appetite changes (too much hunger or loss of appetite)	Ingestion of undercooked pork meat containing cysticerci or ingestion of Taenia solium eggs via fecally contaminated food or water
	Cysticercosis (Neurocysticercosis)	Cysticerci in the brain may cause increased cranial pressure, convulsions and altered mental states	

Infection or presence of pathogen may be identified by:

- Histology
- Microscopy
- PCR
- Serology: e.g. ELISA

2. INTENDED USE

The Taenia solium IgG ELISA is intended for the qualitative determination of IgG class antibodies against Taenia solium in human serum or plasma (citrate, heparin).

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
Microtiterplates are coated with specific antigens to bind corresponding antibodies of the sample. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugate is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of specific antibodies in the sample. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA Microtiterplate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate:** 12 break-apart 8-well snap-off strips coated with Taenia solium antigens; in resealable aluminium foil.
- **IgG Sample Dilution Buffer:** 1 bottle containing 100 mL of phosphate buffer (10 mM) for sample dilution; pH 7.2 ± 0.2; coloured yellow; ready to use; white cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).
- **Stop Solution:** 1 bottle containing 15 mL sulphuric acid, 0.2 mol/L; ready to use; red cap.
- **Washing Buffer (20x conc.):** 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2, for washing the wells; white cap.
- **Conjugate:** 1 bottle containing 20 mL of peroxidased Protein A in phosphate buffer (10 mM); coloured blue; ready to use; black cap.
- **TMB Substrate Solution:** 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB), < 0.1 %; ready to use; yellow cap.
- **Positive Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; red cap; ≤ 0.02% (v/v) MIT.
- **Cut-off Control:** 1 vial containing 3 mL control; coloured yellow; ready to use; green cap; ≤ 0.02% (v/v) MIT.
- **Negative Control:** 1 vial containing 2 mL control; coloured yellow; ready to use; blue cap; ≤ 0.0015% (v/v) CMIT/ MIT (3:1).

For hazard and precautionary statements see 12.1
For potential hazardous substances please check the safety data sheet.

4.2. Materials supplied

- 1 Cover foil
- 1 Instruction for use (IFU)
- 1 Plate layout

4.3. Materials and Equipment needed

- ELISA Microtiterplate reader, equipped for the measurement of absorbance at 450/620 nm
- Incubator 37 °C
- Manual or automatic equipment for rinsing Microtiterplate
- Pipettes to deliver volumes between 10 and 1000 µL
- Vortex tube mixer
- Distilled water
- Disposable tubes

5. STABILITY AND STORAGE

Store the kit at 2...8 °C. The opened reagents are stable up to the expiry date stated on the label when stored at 2...8 °C.

6. REAGENT PREPARATION

It is very important to bring all reagents and samples to room temperature (20…25 °C) and mix them before starting the test run!

6.1. Microtiterplate

The break-apart snap-off strips are coated with Taenia solium antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.

6.2. Washing Buffer (20x conc.)

Dilute Washing Buffer 1 + 19; e. g. 10 mL Washing Buffer + 190 mL distilled water. The diluted buffer is stable for 5 days at room temperature (20…25 °C). In case crystals appear in the concentrate, warm up the solution to 37°C e.g. in a water bath. Mix well before dilution.

6.3. TMB Substrate Solution

The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

7. SAMPLE COLLECTION AND PREPARATION

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the samples should be kept at 2...8 °C; otherwise they should be aliquoted and stored deep-frozen (-70…-20 °C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing.
Heat inactivation of samples is not recommended.

7.1. Sample Dilution

Before assaying, all samples should be diluted 1+100 with IgG Sample Dilution Buffer. Dispense 10 µL sample and 1 mL IgG Sample Dilution Buffer into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

8. ASSAY PROCEDURE

Please read the instruction for use carefully **before** performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend increasing the washing steps from three up to five and the volume of Washing Buffer from 300 µL to 350 µL to avoid washing effects. Pay attention to chapter 12. Prior to commencing the assay, the distribution and identification plan for all samples and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder.

Perform all assay steps in the order given and without any delays.

A clean, disposable tip should be used for dispensing each standard/control and sample.

Adjust the incubator to 37 ± 1 °C.

1. Dispense 100 µL standards/controls and diluted samples into their respective wells. Leave well A1 for the Substrate Blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37 ± 1 °C.**
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300 µL of Washing Buffer. Avoid overflows from the reaction wells. The interval between washing and aspiration should be > 5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL Protein A conjugate into all wells except for the Substrate Blank well A1.
6. **Incubate for 30 min at room temperature (20...25 °C).** Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL TMB Substrate Solution into all wells.
9. **Incubate for exactly 15 min at room temperature (20...25 °C) in the dark.** A blue colour occurs due to an enzymatic reaction.
10. Dispense 100 µL Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the Stop Solution.

8.1. Measurement

Adjust the ELISA Microtiterplate reader **to zero** using the **Substrate Blank**.

If - due to technical reasons - the ELISA Microtiterplate reader cannot be adjusted to zero using the Substrate Blank, subtract its absorbance value from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at **450 nm** and record the absorbance values for each standard/control and sample in the plate layout.

Bichromatic measurement using a reference wavelength of 620 nm is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Run Validation Criteria

In order for an assay run to be considered valid, these Instructions for Use have to be strictly followed and the following criteria must be met:

- **Substrate Blank**: Absorbance value **< 0.100**
- **Negative Control**: Absorbance value **< 0.200 and < Cut-off**
- **Cut-off Control**: Absorbance value **0.150 – 1.300**
- **Positive Control**: Absorbance value **> Cut-off**

If these criteria are not met, the test is not valid and must be repeated.

9.2. Calculation of Results

The Cut-off is the mean absorbance value of the Cut-off Control determinations.

Example: Absorbance value Cut-off Control 0.44 + absorbance value Cut-off control 0.42 = 0.86 / 2 = 0.43

Cut-off = 0.43

9.2.1. Results in Units [NTU]

$$\frac{\text{Sample (mean) absorbance value} \times 10}{\text{Cut-off}} = \text{[NovaTec Units = NTU]}$$

Example: $\frac{1.591 \times 10}{0.43} = 37$ NTU

9.3. Interpretation of Results

Cut-off	10 NTU	-
Positive	> 11 NTU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 NTU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as **negative**.
Negative	< 9 NTU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

10. SPECIFIC PERFORMANCE CHARACTERISTICS

The results refer to the groups of samples investigated; these are not guaranteed specifications.

For further information about the specific performance characteristics please contact NovaTec Immundiagnostica GmbH.

10.1. Precision

Intraassay	n	Mean (E)	CV (%)
#1	24	0.460	4.89
#2	24	0.782	6.40
#3	24	0.790	8.29

Interassay	n	Mean (NTU)	CV (%)
#1	12	17.20	5.23
#2	12	20.32	7.84
#3	12	3.70	13.87

10.2. Diagnostic Specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte.
It is 100% (95% confidence interval: 96.67% - 100%).

10.3. Diagnostic Sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte.
It is 100% (95% confidence interval: 78.2% - 100%).

10.4. Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

10.5. Cross Reactivity

Cross reaction of the antigens with antibodies against Echinococcus and Entamoeba is possible.

11. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the sample may affect the absorbance values.

12. PRECAUTIONS AND WARNINGS

- The test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.
- Only for in-vitro diagnostic use.
- All materials of human or animal origin should be regarded and handled as potentially infectious.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive.
- Do not interchange reagents or Microtiterplates of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and standard/control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense reagents without splashing accurately into the wells.
- The ELISA is only designed for qualified personnel following the standards of good laboratory practice (GLP).
- For further internal quality control each laboratory should additionally use known samples.

12.1. Safety note for reagents containing hazardous substances

Reagents may contain CMIT/MIT (3:1) or MIT (refer to 4.1)
Therefore, the following hazard and precautionary statements apply.

Warning

H317	May cause an allergic skin reaction.
P261	Avoid breathing spray.
P280	Wear protective gloves/ protective clothing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362+P364	Take off contaminated and Wash it before reuse.

Further information can be found in the safety data sheet.

12.2. Disposal Considerations

Residues of chemicals and preparations are generally considered as hazardous waste. The disposal of this kind of waste is regulated through national and regional laws and regulations. Contact your local authorities or waste management companies which will give advice on how to dispose hazardous waste.

13. ORDERING INFORMATION

Prod. No.: TAEG0420 Taenia solium IgG ELISA (96 Determinations)

DEUTSCH

1. EINLEITUNG

Taenia solium (Schweinebandwurm) ist ein weltweit verbreiteter Bandwurm (Cestode) von etwa 2-7m Länge. Er lebt im Dünndarm des Menschen und verschiedener Tiere (Affe, Hamster). Der Bandwurm produziert Proglottiden (< 1.000, jede Proglottide enthält 50.000 Eier), die reifen, sich vom Bandwurm abschnüren, zum Anus wandern oder mit dem Stuhl transportiert werden. Die in den Proglottiden enthaltenen Eier sind in der Umwelt Monate bis Jahre überlebensfähig. Nach der Aufnahem der Eier durch einen geeigneten Zwischenwirt (Schweine und andere Tiere inkl. Mensch) reifen die Larven schnell heran und werden freigesetzt. Sie durchdringen in die Darmwand und wandern in die Muskulatur, das Gehirn, Leber und andere Wirtsgewebe, wo sich die Zystizerken entwickeln. Im menschlichen Darm entwickelt sich ein Zystikerkus innerhalb von 2 Monaten zu einem adulten Bandwurm. Dieser kann bis zu 25 Jahre überleben. Das Hauptsymptom einer milden Taeniasis ist die passive Passage von Proglottiden. Das wichtigste Risiko einer durch T. solium ausgelöste Erkrankung ist die Zystizerkose, die sich im Auge oder ZNS entwickeln kann. Die Prävalenz für eine Infektion ist in armen Gemeinschaften, wo Mensch und Schwein in engem Kontakt zueinander leben und ungares Schweinefleisch gegessen wird, größer. In islamischen Ländern ist die Erkrankung sehr selten.

Spezies	**Erkrankung**	**Symptome (z.B.)**	**Infektionsweg**
Taenia solium	Taeniasis	Bauchschmerzen; Übelkeit; Schwäche und Müdigkeit; Gewichtsverlust; Flatulenz (Gas); Durchfall oder Verstopfung; Änderungen von Appetit (Hunger oder Appetitlosigkeit)	Aufnahme von Bandwurmeiern über Unzureichend gekochter Schweinefleisch oder durch Fäkalien kontaminierte Nahrung oder Wasser
	Zystizerkose (Neurocysticercose)	Cysticerci im Gehirn kann erhöhten Schädeldruck, Krämpfe und veränderte geistige Zustände verursachen	

Nachweis des Erregers bzw. der Infektion durch:

- Histologie
- Mikroskopie
- PCR
- Serologie: z.B. ELISA

2. VERWENDUNGSZWECK

Der Taenia solium IgG ELISA ist für den qualitativen Nachweis spezifischer IgG-Antikörper gegen Taenia solium in humanem Serum oder Plasma (Citrat, Heparin) bestimmt.

3. TESTPRINZIP

Die qualitative immunenzymatische Bestimmung von spezifischen Antikörpern beruht auf der ELISA (Enzyme-linked Immunosorbent Assay) Technik.
Die Mikrotiterplatten sind mit spezifischen Antigenen beschichtet, an welche die korrespondierenden Antikörper aus der Probe binden. Ungebundenes Probenmaterial wird durch Waschen entfernt. Anschließend erfolgt die Zugabe eines Meerettich-Peroxidase (HRP) Konjugates. Dieses Konjugat bindet an die an der Mikrotiterplatte gebundenen spezifischen Antikörper. In einem zweiten Waschschritt wird ungebundenes Konjugat entfernt. Die Immunkomplexe, die durch die Bindung des Konjugates entstanden sind, werden durch die Zugabe von Tetramethylbenzidin (TMB)-Substratlösung und eine resultierende Blaufärbung nachgewiesen.
Die Intensität des Reaktionsproduktes ist proportional zur Menge der spezifischen Antikörper in der Probe. Die Reaktion wird mit Schwefelsäure gestoppt, wodurch ein Farbumschlag von blau nach gelb erfolgt. Die Absorption wird bei 450/620 nm mit einem Mikrotiterplatten-Photometer gemessen.