TECO

CE

# KONFORMITÄTSERKLÄRUNG
# DECLARATION OF CONFORMITY

Doc#001-01/06-2022

Hersteller / Manufacturer: **TECO Medical Instruments Production and Trading GmbH**

Adresse / Address: **Dieselstrasse 1, 84088 Neufahrn, Germany**

Marktakteur / Actor ID SRN: **DE-MF-000022642** https://ec.europa.eu

Die hier benannten Produkte der generischen Produktgruppe erfüllen die Anforderungen der aufgeführten Verordnungen, Richtlinien und Normen. Im Falle eigenmächtiger Veränderungen am Produkt oder der nicht bestimmungsgemäßen Verwendung verliert diese Erklärung ihre Gültigkeit.

Diese Konformitätserklärung wird unter der alleinigen Verantwortung des Herstellers ausgestellt.

**BASIS UDI-DI 426018278CMX81152**

IVD - halb-automatische Blutgerinnungsmessgeräte - Handelsbezeichnung, Typ, Kat.-Nr.
IVD - semi-automated Coagulation Systems - trade name, type, model, Cat.-No.

## Coatron X Eco / Coatron X Pro / Coatron X Top

**81 101 10** **81 101 20** **81 101 40**

The products of the generic product group named here fulfil the requirements of listed regulations, directives and standards. In the case of unauthorised modifications to the product or use not in accordance with the intended purpose, this declaration becomes invalid.

This declaration of conformity is issued under the sole responsibility of the manufacturer.

**Verordnung (EU) 2017/746**
für in-vitro Diagnostika-IVDR
und dem harmonisierten Standard am 2022-05-12:

Risikoklassifizierung gemäß Artikel 47–Anhang VIII
**Regel 5 b – „Klasse A "**

Konformitätsbewertungsverfahren gemäß:
(EU) 2017/746 Artikel 17 (Anhang II+III)

Angewandte Normen zur Sicherstellung der grundlegenden Anforderungen an Leistung und Sicherheit:

EN ISO 18113-3:2011
DIN EN 62304:2018
DIN EN 62366-1
DIN EN 62366-1:2017
DIN EN 61326-1:2013
DIN EN 55011:2009 + A1:2010
IEC 61010-1:2010, AMD1:2016
IEC 61010-2-101:2015
IEC 61010-1:2010

Richtlinie 2011/65/EU RoHS III
(incl. (EU) 2015/863) - DIN EN IEC 63000

QM-System gemäß (EU) 2017/746 Art.10(8)
angewandter Standard: EN ISO 13485:2021

**Regulation (EU) 2017/746**
for In-vitro diagnostic medical devices
and it's harmonized standard at 2022-05-12:

Risk classified according to article 47 annex VIII
**Rule 5 b – "Class A"**

Conformity assessment procedure in accordance with:
(EU) 2017/746 Article 17 (annex II+III)

Standards applied to ensure the essential requirements for performance and safety:

EN ISO 18113-3:2011
DIN EN 62304:2018
DIN EN 62366-1
DIN EN 62366-1:2017
DIN EN 61326-1:2013
DIN EN 55011:2009 + A1:2010
IEC 61010-1:2010, AMD1:2016
IEC 61010-2-101:2015
IEC 61010-1:2010

Directive 2011/65/EU RoHS III
(incl. (EU) 2015/863 - DIN EN IEC 63000

QM-Systems in accordance with (EU) 2017/746 art.10(8)
Applied standard procedure: EN ISO 13485:2021

Ort und Datum der Unterzeichnung:
Place and date of issue: Neufahrn, 2022-06-21

Medical Instruments TECO Production+Trading GmbH

Matthias Dieckmann
General Manager

Christian Hötzl
Verantwortliche Person / PRRC

Digitally signed by Monastîrschii Viorica
Date: 2024.02.02 11:26:59 EET
Reason: MoldSign Signature
Location: Moldova

# KONFORMITÄTSERKLÄRUNG

# DECLARATION OF CONFORMITY

Doc#100/07-2021

Wir / *We*

**TECO Medical Instruments Production and Trading GmbH**
Name des Herstellers / Manufacturer's *name*
**Dieselstrasse 1, 84088 Neufahrn, Germany**
Anschrift / *Address*

erklären in alleiniger Verantwortung, dass die unten gelisteten IVD Zubehör Produkte:
*declare under our own responsibility, that the IVD accessories products, listed below:*

| | |
|---|---|
| Doppelküvette / *Double cuvette* | Ref. 19 000 02 |
| Einzelküvette / *Single cuvette* | Ref. 20 000 02, 24 100 00 |
| 4-fach Küvette / *Cuvette 4 pos/ea* | Ref. 80 521 10 |
| 6-fach Küvette / *Cuvette 6 pos/ea* | Ref. 80 560 00 |
| 6-fach Küvette (micro) / *Cuvette 6 pos/ea (micro)* | Ref. 80 570 00 |

allen anwendbaren Anforderungen folgender Richtlinien entsprechen:

*meet all applicable requirements of:*

1. Richtlinie 98/79/EG über In-vitro Diagnostika und ihrem Zubehör, klassifiziert gemäß Artikel 9 als: "alle anderen Produkte"- im Sinne von Zubehör zu In vitro Diagnostika gemäß Artikel 1.

*1. Directive 98/79/EC on In-vitro diagnostic medical devices and their accessories, classified according to article 9 as: "all other products" – and in term of accessories for in vitro diagnostics according to artivel 1.*

2. Richtlinie 2011/65/EU (RoHS III)

*2. Directive 2011/65/EU (RoHS III)*

Das QM-System des Herstellers ist zertifiziert nach:

*The QM-system of the manufacturer is certified for:*

**EN ISO 13485:2016**

***EN ISO 13485:2016***

Konformitätsbewertungsverfahren gemäß:

*Conformity assessment procedure according to:*

Gemäß Anhang III der Richtlinie 98/79/EG

*According to Annex III of Directive 98/79/EC*

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Ort und Datum der Unterzeichnung:
Place and date of issue:

Neufahrn, 27.07.2021
Neufahrn, July 27, 2021

Medical Instruments TECO Production+Trading GmbH

Matthias Dieckmann
General Manager

TECO
MEDICAL INSTRUMENTS
PRODUCTION+TRADING GMBH
Dieselstraße 1
D-84088 Neufahrn N.B.
fon: +49-8773/707 80-0
fax: +49-8773/707 80-29

Neufahrn, 26/04/2018

**TO WHOM IT MAY CONCERN**

We confirm that the instruments Coatron X Eco, Coatron X Pro and Coatron X Top have a closed cuvette system. Cuvettes have to be purchased with voucher identification code from TECO GmbH.

Medical Instruments
TECO
Production+Trading GmbH

Christian Hoetzl
General Manager
TECO Germany

# KONFORMITÄTSERKLÄRUNG
# DECLARATION OF CONFORMITY

Doc#200/08-2022

| | |
|---|---|
| Hersteller / Manufacturer: | **TECO Medical Instruments Production + Trading GmbH** |
| Adresse / Address: | **Dieselstrasse 1, 84088 Neufahrn, Germany** |
| Marktakteur / Actor ID SRN: | **DE-MF-000022642** https://ec.europa.eu |

Wir erklären hier für die im Anhang A ( Seite 2 – 23 IVD Produkte) spezifizierten Produkte dass sie gemäß der Richtlinie für In-vitro-Diagnostika Medizinprodukte 98/79/EC klassifiziert sind als allgemeine IVD.

Diese Konformitätserklärung wird unter der alleinigen Verantwortung des Herstellers i.V.m. Artikel 110 Abs.3 und Abs.4 der Verordnung (EU) 2017/746 und des § 8 Abs.1 des Medizinprodukte-Durchführungsgesetzes, in der jeweils geltenden Fassung, ausgestellt.

Im Falle eigenmächtiger Veränderungen am Produkt oder der nicht bestimmungsgemäßen Verwendung verliert diese Erklärung ihre Gültigkeit.

We declare herewith for the products specified in Annex A ( page 2 - 23 IVD products) that they are classified as general IVD according to the In Vitro Diagnostic Medical Devices Directive 98/79/EC.

This declaration of conformity is issued under the sole responsibility of the manufacturer in according to article 110 para.3 and para.4 of Regulation (EU) 217/746 and section 8 para.1 of the Medical Device Law Implementing Act.

In case of unauthorised modifications to the products or un-intended use, this declaration loses its validity.

| Sie entsprechen den anwendbaren Anforderungen der Richtlinie: | They meet applicable requirements of: |
|---|---|
| Richtlinie 98/79/EG über In-vitro-Diagnostika klassifiziert gemäß Artikel 9 als "alle anderen Produkte" | Directive 98/79/EC on in-vitro-diagnostic medical devices classified according to article 9 as „all other products" |
| Die Qualitätssicherung entspricht den Anforderungen der Richtlinie 98/79/EG über In-vitro-Diagnostika für diese Art von Produkten. | The Quality Assurance is in accordance with the requirements of Directive 98/79/EC on in-vitro-diagnostic medical devices for those kind of products. |
| Der implementierte QM-Prozess entspricht der EN ISO 13485:2021 | The implemented QM Process complies with EN ISO 13485:2021 |
| Die vorstehende Konformitätserklärung ist gültig für alle Chargen dieser Produkte, die nach dem Datum der Unterzeichnung in Verkehr gebracht wurden. | The above mentioned declaration of conformity is valid for all lots of this product, which are distributed after the date of signature. |
| Das Konformitätsbewertungsverfahren entspricht Anhang III der Richtlinie 98/79/EG über In-vitro-Diagnostika für diese Art von Produkten. | The conformity assessment procedure complies with Annex III of Directive 98/79/EC on in-vitro-diagnostic medical devices for those kind of products. |

Ort und Datum der Unterzeichnung:
Place and date of issue: Neufahrn, 2022-08-31

Medical Instruments TECO Production+Trading GmbH

Christian Hötzl
Verantwortliche Person / PRRC



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Doc#200/08-2022

# KONFORMITÄTSERKLÄRUNG – DECLARATION OF CONFORMITY

Directive 98/79/EC Annex A
Übrige Produkte – Reagenzien für In-vitro-Diagnostika
Other products – Reagents for in vitro diagnostic – general IVD

| Pos. | Article No | Tradename | Unit | Generic Device Term | EMDN / GMDN Code EUDAMED DI |
|---|---|---|---|---|---|
| 1 | A0230-040 | TEClot PT-S (Quick) | 10x4ml PT-S | Prothrombin time ( quick test ) | W0103020101 / 30539 B-PTS-A0230-040X7 |
| 2 | A0230-100 | TEClot PT-S (Quick) | 10x10ml PT-S | Prothrombin time ( quick test ) | W0103020101 / 30539 B-PTS-A0230-100WY |
| 3 | A0260-050 | TEClot PT-B (Owren) | 5x10ml PT-B | Prothrombin time ( quick test ) | W0103020199 / 55986 B-PTB-A0260-050G2 |
| 4 | A0320-050 | TEClot APTT-S | 10x5ml APTT-S | Activated partial thromboplastin time | W0103020102 / 55982 B-APTTS-A0320-050AM |
| 5 | A0401-020 | TEClot TT | 10x2ml TT | Thrombin time / reptilase / batroxbin time | W0103020103 / 55988 B-TT-A0401-0207P |
| 6 | A0511-020 | TEClot FIB | 10x2ml FIB | Fibrinogen assays (factor i) | W0103020201 / 55997 B-FIB-A0511-020N2 |
| 7 | A0511-050 | TEClot FIB | 10x5ml FIB | Fibrinogen assays (factor i) | W0103020201 / 55997 B-FIB-A0511-050NB |
| 8 | C1010-020 | TEChrom AT | 6x6ml reagent FXa 3x3 ml substrate | Antithrombin | W0103020602 / 56156 B-AT-C1010-020HL |
| 9 | D2010-012 | Red D-Dimer | 3x4ml latex 3x7ml reaction buffer | D-Dimer | W0103020503 / 47349 B-DD-D2010-0126W |
| 10 | D2020-005 | Blue D-Dimer LC | 1x5ml latex LC 1x7ml reaction buffer | D-Dimer | W0103020503 / 47349 B-DD-D2020-0057E |
| 11 | P8001-010 | TECal N | 10x1ml | Calibration plasma for haemostasis | W0103020701 / 45786 B-CAL-P8001-005X8 |
| 12 | P8200-005 | TECal DD | 5x1ml | Calibration plasma for haemostasis | W0103020701 / 47348 B-CAL-P8200-005XX |
| 13 | P6001-010 | TEControl N | 10x1ml | Control plasma for haemostasis | W0103020702 / 30590 B-CTRL-P6001-010H7 |
| 14 | P6101-010 | TEControl A | 10x1ml | Control plasma for haemostasis | W0103020702 / 30590 B-CTRL-P6101-010HQ |
| 15 | P6201-010 | TEControl A Plus | 10x1ml | Control plasma for haemostasis | W0103020702 / 30590 B-CTRL-P6201-010J9 |
| 16 | P5001-010 | TEClot Factor II | 10x1ml | Coagulation factor ii ( prothrombin ) | W0103020202 / 30542 B-FAC-II-P5001-010ML |
| 17 | P5101-010 | TEClot Factor V | 10x1ml | Coagulation factor v | W0103020204 / 30544 B-FAC-V-P5101-010AN |
| 18 | P5201-010 | TEClot Factor VII | 10x1ml | Coagulation factor vii | W0103020205 / 30545 B-FAC-VII-P5201-0107B |
| 19 | P5301-010 | TEClot Factor VIII | 10x1ml | Coagulation factor viii | W0103020207 / 30547 B-FAC-VIII-P5301-01097 |
| 20 | P5401-010 | TEClot Factor IX | 10x1ml | Coagulation factor ix | W0103020208 / 30548 B-FAC-IX-P5401-0106C |
| 21 | P5501-010 | TEClot Factor X | 10x1ml | Coagulation factor x | W0103020209 / 30549 B-FAC-X-P5501-010EQ |
| 22 | P5601-010 | TEClot Factor XI | 10x1ml | Coagulation factor xi | W0103020210 / 30551 B-FAC-XI-P5601-010A8 |
| 23 | P5701-010 | TEClot Factor XII | 10x1ml | Coagulation factor xii | W0103020211 / 30552 B-FAC-XII-P5701-010CJ |

(Recital 23 of Directive 98/79/EC on In Vitro Diagnostics Medical Devices) - Annex A - general IVD



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**TO WHOM IT MAY CONCERN**
To any governmental departments,
registration and/or trade offices in MOLDOVA

**Distribution Authorisation Letter**

This letter confirms that **Sanmedico**
**Mun. Chisinau**
**Str. Petricani 88/1 of. 10**
**Republica MOLDOVA**

is the **legal, exclusive and sole** representative of **TECO Medical Instruments Production + Trading GmbH, Dieselstr. 1, 84088 Neufahrn NB, Germany,** for the territory of **MOLDOVA** only for all TECO products listed below. **Sanmedico** may participate in public and private tenders, providing sales to all TECO customers in the territory. We as manufacturer certify that our warranty is duly passed to the purchaser through **Sanmedico** for the price, delivery schedules and the specifications of the published literature, catalogues and fully covering the commodities offered.

**Sanmedico** will provide the following information to TECO GmbH when so required in relation to its market surveillance activities:
Reporting of incidents to TECO must take place within 3 working days
Serial number of the device, exact location of the device and the user.

Validity: January 1st, 2023 to December 31st, 2024

Termination: Confirmation ends automatically on Dec. 31st of 2024 and must be then renewed.

Products:

- Coatron M1 — Semi-automated 1-channel Coagulometer (out of production)
- Coatron M2 — Semi-automated 2-channel Coagulometer (out of production)
- Coatron X Eco — Semi-automated 1-channel Coagulometer
- Coatron X Pro — Semi-automated 2-channel Coagulometer
- Coatron X Top — Semi-automated 4-channel Coagulometer
- Coatron A4 — Fully automated Coagulometer, 4 optic channels
- Coatron A6 — Fully automated Coagulometer, 6 optic channels
- Coatron A6 plus — Fully automated Coagulometer, 6 optic channels

all instruments with complete accessory, consumables and spare parts

- Hemostasis Reagents — Complete product line

This document is signed in Neufahrn, Germany, on January 18th, 2023

TECO Medical Instruments Production+Trading GmbH

Christian Hoetzl

Medical Instruments TECO Production+Trading GmbH

Certificate -Bescheinigung

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# CERTIFICATE OF TRAINING

**Vitalie Goreacii**
General manager of
Sanmedico
Chisinau
Republic of Moldava

**have participated with success at the training session supervised by TECO GmbH, Germany for following instruments:**

**Coatron A series**

- **Installation**
- **Application**
- **General use, also in combination with TECAM**
- **Maintenance**
- **Troubleshooting**
- **After Sales Service**

Training details:

| | |
|---|---|
| Supervisor: | Chr. Baumgartner, Director RD of TECO |
| Device | Coatron A4 + A6, Inhouse Master Device |
| Place: | Laboratories of TECO |
| Date: | May 5th 2023 |

TECO
Dipl.-Ing. Univ.
Christian Baumgartner

Dipl.-Ing. Univ. (TUM)
**Christian Baumgartner**
Director R&D

| | | |
|---|---|---|
| Current issue date: | 10 November 2022 | Original approval(s): |
| Expiry date: | 9 November 2025 | ISO 13485 - 10 November 2022 |
| Certificate identity number: | 10479697 | |

# Certificate of Approval

This is to certify that the Management System of:

## TECO Medical Instruments, Production + Trading GmbH

Dieselstr. 1, 84088 Neufahrn, Germany

has been approved by LRQA to the following standards:

**ISO 13485:2016**

Approval number(s): ISO 13485 – 00038268

**The scope of this approval is applicable to:**

Design, development, manufacturing, storage and sales of coagulation instruments and in-vitro-diagnostic reagents used in the hemostaseology and coagulation.

**Paul Graaf**

Area Operations Manager, Europe

Issued by: LRQA Limited

UKAS MANAGEMENT SYSTEMS 0001

# TEClot PT-S

CE IVD REF **A0230-010, A0230-040, A0230-100,**

## Intended Use
This product is used for the determination of prothrombin time (PT) in plasma according to Quick[1,2]. The test is sensitive to the extrinsic pathway coagulation factors II,V,VII,X and fibrinogen and therefore used for oral anticoagulant therapy with Vitamin-K inhibitors like Warfarin or Marcumar and also for the quantitative determination of extrinsic coagulation factors. The PT measures the extrinsic clotting time (factor VII activation) of test plasma after the addition PT reagent.

## Contents & Determinations

| Product | TEClot PT-S | TEClot PT-S | TEClot PT-S |
|---|---|---|---|
| Cat.No. | A0230-010 | A0230-040 | A0230-100 |
| PT-S Reagent* | 5x2 mL | 10x4 mL | 10x10 mL |

Determinations

| | | | |
|---|---|---|---|
| Coatron M** | 200 Det. | 800 Det. | 2000 Det. |
| Coatron A4 | 100 Det. | 400 Det. | 1000 Det. |
| Coatron A6 | 200 Det. | 800 Det. | 2000 Det. |

*contains an extract of Rabbit brain with buffer, stabilizers and Calcium chloride.
**Micro method (75µL in total)

## Preparation
Reconstitute with high purity water with the volume stated on the vial label.

| A0230-010 | A0230-040 | A0230-100 |
|---|---|---|
| 2 mL | 4 mL | 10 mL |

Let stand at room temperature with occasional swirling for at least 15 min. Then place reagent into instrument and let incubate for further 15 min. The reagent sediments and must be swirled before each testing. On Coatron instruments, you can use a mixing bar for this.

## Storage & Stability
Unopened reagents are stable until the expiration date shown on the label stored at 2°-8°C. Opened reagent:

| | 2-8 °C | 20-25 °C | 37°C |
|---|---|---|---|
| PT Reagent | 5 days | 36 hours | 8 hours |

## Precautions
Avoid contact with skin and eyes. Wear suitable protective clothing. Dispose components in compliance with local regulations for infectious material. All components are checked for HIV, HBV, HCV. However products from human blood should be considered as potentially infectious.

## Specimen collection and storage[4]
1. Obtain venous blood by clean vein puncture.
2. Immediately mix 9 parts blood with 1 part 3.2% sodium citrate (0.105M) and mix well
3. Centrifuge the specimen at 1500g for 10 min. (platelet < 10000/µL)
4. Separate plasma after centrifugation and store in plastic or siliconised glass tube.
5. Use plasma within 4 hours, otherwise store frozen and thaw just prior to use.

Stability of plasma: 4h at 18-26°C 8h at 2-8° 30d at -20°C 6m at -70°C

## Procedure
**A. Automated Method: Coatron A**

| Prothrombin Time | | A4 | | A6 | |
|---|---|---|---|---|---|
| PAT | Patient | 50µl | CP1 | 25µl | CP1 |
| BUF | IBS Buffer | 0µl | P39 | 0µl | P79 |
| CLR | - | 0µl | - | 0µl | - |
| DP | - | 0µl | P00 | 0µl | P00 |
| R0 | - | 0µl | P00 | 0µl | P00 |
| R1 | - | 0µl | P00 | 0µl | P00 |
| R2 | PT Reagent | 100µl | P25 | 50µl | P46 |

| | A4 | A6 |
|---|---|---|
| Incubation | 0s | |
| Maxtime | 120s | |
| Unit | 251 | |
| Method | Coag | |
| Math | log XY | |
| CT-Mech | No | |
| Deadtime | 7s | |

| | A4 | A6 |
|---|---|---|
| SENS | 2 | |
| POINTS | 4 | |
| MIX | No | |
| Clean | 0 | 0 |
| Multi | 1 | 3 |
| S-Corr | 0% | |
| T-Corr | 30% - 4s | |

**B. Manual Method: Coatron M system**
1. Incubate PT reagent at 37°C for at least 10 minutes
2. Pipette **25 µl of sample** into a test cuvette. Incubate at 37°C for 1-2 minutes.
3. Add **50 µl of PT reagent** (37°C) and simultaneously start test.
4. Record the clotting time in seconds.

For other instrument, please refer to your instrument manual for more detailed instrument specific instructions.

## Expected Results
Typical seconds: 11 – 18 sec
Normal range: 70 - 130% 0.85 – 1.15 INR

However results are influenced by instruments, technique, calibration etc. Each laboratory is recommended to establish its own range on the specific instrument used.

## Standardisation and Calibration
The PT result is expressed as seconds or activity (% Quick) or INR (International Normalised Ratio).

INR results:
were calculated from normal time and ISI value (international sensitivity index). First is obtained by running fresh plasma from a pool of healthy individuals. The ISI value is stated in the LOT specific certificate of analysis.

$$INR = \left(\frac{Patient\ PT}{Normal\ PT}\right)^{ISI}$$

Activity % (Quick) result:
were calcaluted from a calibration curve, which is prepared from reference plasma (e.g. TECAL N) and dilutions in saline solution like 0.9% $NaCl_2$ or TECLOT IBS buffer. At least three or more calibration points are recommended. The calibration curve must be confirmed with control plasma in normal and abnormal range.

| % of normal | 100%* | 50% | 25% | 12,5%** |
|---|---|---|---|---|
| diluted in saline | not dil. | 1+1 | 1+3 | 1+7 |

* The median of at least 21 healthy individuals is defined as 100%.[5]
**12.5% dilution may cause "+++" results in same cases, because the level of fibrinogen is too high diluted for optical detection.

## Quality Control
TEControl or other commercial control plasma should be used for reliable quality control of performance at a frequency in accordance with good laboratory practice (GLP). TEControl can be frozen one time after reconstitution. 120-150 µl stored in closed polypropylen tubes at -20°C is stable for 30 days

## Limitations
Great care must be taken to minimize variations which may occur by seemingly insignificant factors.
A. Specimen Collection. AVOID:
1. Use only plastic tubes or siliconised glass.
2. Delayed mixing of blood with anticoagulant.
3. Contamination with tissue thromboplastin.
4. Improper ratio of anticoagulant with blood.
5. Hemolyzed, icteric or lipemic samples may interfere optical systems

B. Laboratory Techniques
1. Perform tests at 37°C.
2. Use only high purity water.
3. Optimum pH is 7.0-7.5.
4. ISI value is not constant within the first 30 min after reconstitution.
5. Reagent sediments and must be swirled before each testing.

## Performance Characteristics
**Typical performance on instrument Coatron M4**

| **Precision:** | CV% (within run) | CV% (inter-runs) |
|---|---|---|
| Normal control | < 3,0 | < 5,0 |
| Abnormal control | < 3,0 | < 5,0 |

## Warranty
This product is warranted to perform in accordance with its labelling and literature. TECO disclaims any implied warranty of merchantability or fitness for any other purpose, and in no event will TECO be liable for any consequential damages arising out of aforesaid express warranty.

## References

1. Quick, A.J., The Hemorrhagic Diseases and the Physiology of Hemostasis. Charles C. Thomas: Springfield, IL. 1942.
2. Quick, A.J., Hemorrhagic Diseases. Lea and Febiger: Philadelphia. 1957.
3. Miale, J.B., Laboratory Medicine-Hematology, 4th Edition. C.V. Mosby: St. Louis. 1972.
4. National Committee for Clinical Laboratory Standards: Guidelines for the Standardized Collection, Transport and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays.
5. Besselaar A M H P van den, Lewis SM, Mannucci P n Poller L. 1993. Status of present and candidate International Reference Preparations (IRP) of thromboplastin for prothrombin time. Thromb Hemostas 69; 85
6. Besselaar A M H P van den. 1991. The significance of the International Normalized Ratio (INR) for oral anticoagulant therapy. H17CC 3; 146153.


Symbol keys

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Expiry date | IVD | In Vitro Diagnostica | | Biological hazard | REF | Catalogue Number | AQUA DEST. | Reconstitute with dest. water | | Consult accompanying documents |
| | Store at 2-8°C | CE | EU conformity | | Manufacturer | LOT | Lot. Number | | Ready to use | EC REP | Authorized Representative |


TECO GmbH, Dieselstr. 1, 84088 Neufahrn NB Germany

# TEClot FIB

IVD REF **A0501-010, A0501-025, A0511-020, A0511-050**

## Intended Use

The TEClot FIB is intended for the quantitative determination of fibrinogen in human plasma according to method developed by Clauss.[1] Levels of fibrinogen can increase as a result of inflammation, pregnancy or oral contraceptive use[2]. Decreased levels can be found in certain states such as liver disease and DIC. Congenital deficiencies include afibrinogenaemia (no detectable fibrinogen), hypofibrinogenaemia (<1 mg/ml) and dysfibrinogenaemia (abnormal fibrinogen molecule).

## Contents & Preparation

| Product | TEClot FIB Kit-10 | TEClot FIB Kit-25 | TEClot FIB | TEClot FIB |
|---|---|---|---|---|
| Cat.No. | A0501-010 | A0501-025 | A0511-020 | A0511-050 |
| Thrombin Reagent | 5x2 mL | 5x5 mL | 10x2 mL | 10x5 mL |
| IBS Buffer | 1x125 mL | 1x125 mL | - | - |
| TECal Normal | 1x1 mL | 1x1 mL | - | - |
| TEControl A | 1x1 mL | 1x1 mL | - | - |

Determinations

| | | | | |
|---|---|---|---|---|
| Coatron M* | 400 Det. | 1000 Det. | 800 Det. | 2000 Det. |
| Coatron A4 | 200 Det. | 500 Det. | 400 Det. | 1000 Det. |
| Coatron A6 | 200 Det. | 500 Det. | 400 Det. | 1000 Det. |

*Micro method (75µL in total)

1. Thrombin Reagent:
   Contains bovine thrombin (~80NIH) with stabilizers
   REF: A0501-010/A0511-020: Reconstitute with 2mL purified water
   REF: A0501-025/A0511-050: Reconstitute with 5mL purified water
2. IBS Buffer: Ready to use. Contains Imidazole buffered saline
3. TECal Normal: Reconstitute with 1 mL purified water. Contains citrated human plasma.
4. TEControl A: Reconstitute with 1 mL purified water. Contains citrated human plasma.

Swirl gently after reconstitution and allow standing for 15 minutes at room temperature. Mix well before use. Do not shake.

## Storage & Stability

Unopened reagents are stable until the expiration date shown on the label stored at 2°-8°C. Opened reagent:

| | | | |
|---|---|---|---|
| Thrombin Reagent* | 2-8 °C | 15-25 °C | 37 °C |
| | 12 days | 5 days | 24 hours |
| TEControl or Plasma | 2-8 °C | 15-25 °C | -20 °C |
| | 8 hours | 4 hours | 30 days |

* Reagent must be protected from UV-light and evaporation

## Precautions

Avoid contact with skin and eyes. Wear suitable protective clothing. Dispose components in compliance with local regulations for infectious material.
All components are checked for HIV, HBV, HCV. However products from human blood should be considered as potentially infectious.

## Specimen collection and storage[3]

1. Obtain venous blood by clean vein puncture.
2. Immediately mix 9 parts blood with 1 part 3.2% sodium citrate (0.105M) and mix well
3. Centrifuge the specimen at 1500g for 10 min. (platelet < 10000/µL)
4. Separate plasma after centrifugation and store in plastic or siliconised glass tube.
5. Use plasma within 4 hours, otherwise store frozen and thaw just prior to use.

## Procedure

**A. Automated Method. Coatron A**

| Fibrinogen | | A4 | | A6 | |
|---|---|---|---|---|---|
| PAT | Patient | 10µl | CP1 | 10µl | CP1 |
| BUF | IBS Buffer | 90µl | P39 | 90µl | P79 |
| CLR | - | 0µl | - | 0µl | - |
| DP | - | 0µl | P00 | 0µl | P00 |
| R0 | - | 0µl | P00 | 0µl | P00 |
| R1 | - | 0µl | P00 | 0µl | P00 |
| R2 | Fibrinogen | 50µl | P29 | 50µl | P49 |

| | A4 | A6 |
|---|---|---|
| Incubation | 0s | |
| Maxtime | 120s | |
| Unit | 769 | |
| Method | Coag | |
| Math | log XY | |
| CT-Mech | Yes | |
| Deadtime | 3s | |

| | A4 | A6 |
|---|---|---|
| SENS | 0 | |
| POINTS | 4 | |
| MIX | No | |
| Clean | 1 | 3 |
| Multi | 1 | 1 |
| S-Corr | 0% | |
| T-Corr | 0% | |

**B. Manual Method: Coatron M**

1. Preparation of Standard, Control and Patient Dilutions

| Standard Dilution | Plasma | IBS Buffer |
|---|---|---|
| 1:5 | 200µL Standard | 800µL |
| 1:10 | 500µL 1:5 STD | 500µL |
| 1:20 | 500µL 1:10 STD | 500µL |
| 1:40 | 500µL 1:20 STD | 500µL |
| Patient or Control | 100µL Plasma | 900µL |

2. Pipette **50 µl diluted standard or patient plasma** (1:10) into a test cuvette. Prewarm at 37°C for 1-2 minutes.
3. Add **25 µl Thrombin reagent** and simultaneously start test.

For other instrument, please refer to your instrument manual for more detailed instrument specific instructions.

## Calibration

TECal Normal or other commercially prepared plasma standard in which Fibrinogen has been determined should be used as reference (200-300mg/dL).
Plot the clotting time obtained with each of the FIB standard dilutions on the y-axis against the concentration of FIB (mg/dL) on the x-axis using log-log graph paper. The line of best fit should be determined by linear regression analysis. The fibrinogen in plasma samples can be determined by interpolation from the calibration curve.

## Expected Results

Typical normal results are 180-450 mg/dL[4,5]. However results are influenced by the method of clot detection and can vary from laboratory to laboratory. Each laboratory is recommended to establish its own normal range on the specific instrument used.

## Quality Control

TEControl or other commercial control plasma should be used for reliable quality control of performance at a frequency in accordance with good laboratory practice (GLP). ). TEControl can be frozen one time after reconstitution. 120-150 µl stored in closed polypropylen tubes at -20°C is stable for 30 days

## Limitations

A. Specimen Collection. AVOID:
   1. Use only plastic tubes or siliconised glass.
   2. Delayed mixing of blood with anticoagulant.
   3. Contamination with tissue thromboplastin.
   4. Improper ratio of anticoagulant with blood.
   5. Hemolyzed, icteric or lipemic samples may interfer optical systems

B. Laboratory Techniques
   1. Perform tests at 37°C.
   2. Use only high purity water.
   3. Optimum pH is 7.0-7.5.

## Performance Characteristics

| **Precision:** | CV% (within run) | CV% (inter-runs) |
|---|---|---|
| Normal control | < 5.0 | < 5.0 |
| Abnormal control | < 5.0 | < 10.0 |

(Typical performance on instrument Coatron M4)

## Warranty

This product is warranted to perform in accordance with its labelling and literature. TECO disclaims any implied warranty of merchantability or fitness for any other purpose, and in no event will TECO be liable for any consequential damages arising out of aforesaid express warranty.

## References


1. Clauss, A., Gerinnungsphysiologische Schnellmethode zur bestimmung des Fibrinogens. Acta Haematol., 1957, 17: 237-246.
2. Shaw, T.S.,Assays for Fibrinogen and its Derivatives, CRC Crit. Rev. Clin. Lab. Sci., 1977, 8: 145-192.
3. National Committee for the National Laboratory (NCCLS) Standards: Collection transport and preparation of blood specimens for coagulation testing and performance of coagulation assays. Document H21-A2, vol. 11, No. 23, 1991.
4. Scully, R.E. et al., Normal Reference Laboratory Values, N. Eng. J. Med., 1980, 302(37) : 37-48.
5. Okuno, T. and Selenko, V., Amer. J. Med. Tech., 1972, 38(6) : 196-201.


Symbols key:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Expiry date | IVD | In Vitro Diagnostica | | Biological hazard | REF | Catalogue Number | | Consult accompanying documents |
| | Store at 2-8°C | CE | EU conformity | | Manufacturer | LOT | Lot. Number | EC REP | Authorized Representative |

# TEClot FIB

IVD REF **A0501-010, A0501-025, A0511-020, A0511-050**

## Verwendungszweck

TEClot FIB wird zur quantitativen Bestimmung von Fibrinogen im menschlichen Plasma nach einer von Clauss[1] entwickelten Methode verwendet. Der Fibrinogenpegel kann auf Grund von Entzündungen, Schwangerschaft und dem Gebrauch von Ovulationshemmern ansteigen[2]. Geringere Konzentrationen können bei verschiedenen Krankheiten wie Leberversagen und DIC auftreten. Angeborene Defizite beinhalten Afibrinogenämie (kein auffindbares Fibrinogen), Hypofibrigonämie (<1mg/ml) und Dysfibrinogenämie (abnormale Fibrinogenmoleküle).

## Inhalte und Vorbereitungen

| Produkt | TEClot FIB Kit-10 | TEClot FIB Kit-25 | TEClot FIB | TEClot FIB |
|---|---|---|---|---|
| Kat. Nr. | A0501-010 | A0501-025 | A0511-020 | A0511-050 |
| Thrombin Reagenz | 5x2 mL | 5x5 mL | 10x2 mL | 10x5 mL |
| IBS Puffer | 1x125 mL | 1x125 mL | - | - |
| TECal Normal | 1x1 mL | 1x1 mL | - | - |
| TEControl A | 1x1 mL | 1x1 mL | - | - |

Bestimmungen

| | | | | |
|---|---|---|---|---|
| Coatron M* | 400 Det. | 1000 Det. | 800 Det. | 2000 Det. |
| Coatron A4 | 200 Det. | 500 Det. | 400 Det. | 1000 Det. |
| Coatron A6 | 200 Det. | 500 Det. | 400 Det. | 1000 Det. |

*Mikromethode (75µL insgesamt)

1. Thrombin Reagenz:
   Enthält Rinderthrombin (~80 NIH) mit Stabilisatoren.
   **REF: A0501-010/A0511-020:** mit 2ml hochreinem Wasser anlösen
   **REF: A0501-025/A0511-050:** mit 5ml hochreinem Wasser anlösen
2. IBS Puffer: gebrauchsfertig, 125ml
   Enthält gepufferte Natriumchlorid Lösung, pH 7,3-7,4
3. TECal Normal: Mit 1ml hochreinem Wasser anlösen
   Enthält mit Zitrat versetztes menschliches Plasma.
4. TEControl A: Mit 1ml hochreinem Wasser anlösen
   Enthält mit Zitrat versetztes menschliches Plasma.

Nach der Anlösung vorsichtig leicht schwenken und bei Raumtemperatur 15 Minuten stehen lassen. Vor Gebrauch gut mischen. Nicht schütteln.

## Lagerung und Stabilität

Ungeöffnete Reagenzien sind bei Lagerung zwischen 2-8°C bis zum auf dem Etikett angegebenen Verfallsdatum haltbar. **Geöffnete Reagenzien:**

| | | | |
|---|---|---|---|
| Thrombin Reagenz* | 2-8 °C | 15-25 °C | 37 °C |
| | 12 days | 5 days | 24 Std |
| TEControl oder Plasma | 2-8 °C | 15-25 °C | -20 °C |
| | 8 Std | 4 Std | 30 Std |

* Reagenz muss vor UV-Licht und Verdunstung geschützt werden.

## Vorsichtsmaßnahme

Haut- & Augenkontakt vermeiden. Abfälle gemäß lokaler Richtlinien für infektiöse Materialien entsorgen. Alle Bestandteile wurden auf HIV, HBV und HCV getestet. Trotzdem müssen Produkte aus menschlichem Blut immer als potentiell infektiös behandelt werden.

## Probenentnahme und Lagerung [3]

1. Venöses Blut mittels Venenpunktur unter sauberen Bedingungen entnehmen.
2. Sofort 9 Teile Blut mit einem Teil 3,2% Natriumzitrat (0,105M) gut mischen.
3. Probe bei 1500g 10 Minuten lang zentrifugieren (Thrombozyten <10000µl)
4. Plasma nach der Zentrifugierung entfernen und in einem Röhrchen aus Plastik oder silikonisierten Glas aufbewahren.
5. Plasma innerhalb von 4 Stunden verwenden, andernfalls gefroren lagern und kurz vor Gebrauch auftauen.

## Verfahren

**A. Automatenmethode: Coatron A**

| Fibrinogen | | A4 | | A6 | |
|---|---|---|---|---|---|
| PAT | Patient | 10µl | CP1 | 10µl | CP1 |
| BUF | IBS Buffer | 90µl | P39 | 90µl | P79 |
| CLR | - | 0µl | - | 0µl | - |
| DP | - | 0µl | P00 | 0µl | P00 |
| R0 | - | 0µl | P00 | 0µl | P00 |
| R1 | - | 0µl | P00 | 0µl | P00 |
| R2 | Fibrinogen | 50µl | P29 | 50µl | P49 |

| | A4 | A6 |
|---|---|---|
| Incubation | 0s | |
| Maxtime | 120s | |
| Unit | 769 | |
| Method | Coag | |
| Math | log XY | |
| CT-Mech | Yes | |
| Deadtime | 3s | |

| | A4 | A6 |
|---|---|---|
| SENS | 0 | |
| POINTS | 4 | |
| MIX | No | |
| Clean | 1 | 3 |
| Multi | 1 | 1 |
| S-Corr | 0% | |
| T-Corr | 0% | |

**B. Manuelle Methode: Coatron M**

1. Vorbereitung von Standard-, Kontroll- und Patientenlösungen

| Standardlösung | Plasma | IBS Puffer |
|---|---|---|
| 1:5 | 200µL Standard | 800µL |
| 1:10 | 500µL 1:5 STD | 500µL |
| 1:20 | 500µL 1:10 STD | 500µL |
| 1:40 | 500µL 1:20 STD | 500µL |
| Patient oder Kontrolle | 100µL Plasma | 900µL |

2. 50µl verdünntes Standard- oder Patientenplasma (1:10) in eine Küvette pipettieren. Bei 37°C für 1-2 Minuten erwärmen
3. 25µl Thrombinreagenz hinzufügen und gleichzeitig Test starten.

Wenn Sie ein anderes Gerät verwenden, lesen Sie bitte für genauere Informationen die entsprechende Geräteanleitung.

## Kalibrierung

TECal Normal oder anderes kommerzielles Standardplasma, mit bekanntem Fibrinogengehalt, sollte als Referenz (200-300 mg/dl) verwendet werden. Geben Sie die Gerinnungszeit jeder FIB Standard Lösung auf der Y- Achse gegen die FIB Konzentration (mg/dl) auf der X- Achse an. Verwenden Sie Millimeterpapier. Die Reihe der besten Ergebnisse sollte durch lineare Regressionsanalyse bestimmt werden. Fibrinogen in den Plasmaproben kann durch Interpolation der Kalibrierungskurve bestimmt werden.

## Erwartete Ergebnisse

Typische normale Ergebnisse sind 180-450mg/dl[4,5]. Die Ergebnisse sind jedoch von der Methode, wie die Gerinnungszeit bestimmt wird, abhängig und können von Labor zu Labor variieren. Jedem Labor wird empfohlen, seinen
eigenen normalen Ergebnisbereich auf dem verwendeten Instrument zu erstellen.

## Qualitätskontrolle

TEControl oder anderes kommerzielles Kontrollplasma sollte, um eine gute Qualität sicherzustellen, in regelmäßigen Abständen entsprechend Laborrichtlinien gemessen werden. in regelmäßigen Abständen entsprechend Laborrichtlinien gemessen werden. TEControl kann einmalig wieder eingefroren werden. Hierfür 120-150µL in einem verschließbaren polypropylen Gefäß bei -20°C aufbewahren und innerhalb der nächsten 30 Tage verwenden.

## Beschränkungen

A. Probenvorbereitung. Achten Sie auf:
1. nur Plastikröhrchen oder silikonisiertes Glas verwenden
2. verzögertes Mischen von Blut mit Antikoagulanz vermeiden
3. Kontaminierung mit Gewebethromboplastin vermeiden
4. falsches Verhältnis von Antikoagulanz und Blut vermeiden
5. Hämolytische,lipämische oder ikterische Proben können optische Systeme stören

B. Labortechniken
1. Tests bei 37°C durchführen
2. nur hochreines Wasser verwenden
3. der optimale pH Wert ist 7,0-7,5

## Leistungsdaten

| **Präzision:** | VK% (Einzellauf) | VK% (Mehrfachlauf) |
|---|---|---|
| Normale Kontrolle | < 5.0 | < 5.0 |
| Abnormale Kontrolle | < 5.0 | < 10.0 |

(Typische Leistung beim Gerät Coatron M4)

## Garantie

Es wird garantiert, dass die Wirkungsweise dieses Produktes den Angaben auf der Packung und in der Produktliteratur entspricht. TECO haftet weder für die Verkäuflichkeit oder Eignung dieses Produktes für irgendwelche andere Zwecke noch für irgendwelche Folgeschäden, die sich aus der vorstehenden, expliziten Garantie ergeben.

## Referenzen


1. Clauss, A., Gerinnungsphysiologische Schnellmethode zur bestimmung des Fibrinogens. Acta Haematol., 1957, 17: 237-246.
2. Shaw, T.S.,Assays for Fibrinogen and its Derivatives, CRC Crit. Rev. Clin. Lab. Sci., 1977, 8: 145-192.
3. National Committee for the National Laboratory (NCCLS) Standards: Collection transport and preparation of blood specimens for coagulation testing and performance of coagulation assays. Document H21-A2, vol. 11, No. 23, 1991.
4. Scully, R.E. et al., Normal Reference Laboratory Values, N. Eng. J. Med., 1980, 302(37) : 37-48.
5. Okuno, T. and Selenko, V., Amer. J. Med. Tech., 1972, 38(6) : 196-201.


Erklärung der Symbole:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Verfallsdatum | IVD | In-Vitro Diagnostik | | Biologische Gefahr | REF | Katalog-Nummer | | Begleitpapiere beachten |
| | Bei 2-8°C lagern | CE | EU Konformität | | Hersteller | LOT | Lot. – Nummer | EC REP | Bevollmächtigter |

## Revisions-Übersicht:

| Rev. | am | Änderung durch | Gültig für | Freigabe am | Freigabe durch |
|---|---|---|---|---|---|
| 1 | 5.4.11 | WG | Technoclone FIB | | |
| | Beschreibung: | New box insert for Technoclone FIB. | | | |
| 2 | 21.12.11 | CB | Technoclone FIB | 21.12.11 | CH |
| | Beschreibung: | Neue Stabilitätsangaben. Die Vorgaben wurden dem Technoclone Stability Test Report „TC6E0C.01“ vom 5.5.2010 entnommen. | | | |
| 3 | 11.11.13 | CB | Technoclone FIB | | |
| | Beschreibung: | - Protokoll für A4+A6<br>- Stabilitätsdaten neu | | | |
| 4 | 16.10.17 | AR | Technoclone FIB | 16.10.17 | CH |
| | Beschreibung: | Technoclone Puffer (A0591-090) wird ersetzt durch IBS (A0590-125)<br>(wegen deutlicher Messunterschiede bei Coatron A und X Serie)<br>Werteermittlung für das CoA erfolgt ebenso mit IBS (A0590-125) | | | |
| 5 | 23.01.18 | VG | Technoclone FIB | 23.01.18 | VG |
| | Beschreibung: | Neue Stabilitätsangaben von Technoclone vom Thrombin Reagent. | | | |

# IBS Buffer

CE IVD REF ***A0590-125***

### Intended Use
The IBS Buffer solution is optimally formulated for use on Coagulation Analyzers. Use in accordance with the recommended Operators Manuals for installing and replacing Owrens Veronal Buffer (OVB). The IBS can be used as the diluent for preparing plasma dilutions in the performance of Fibrinogen determinations and Coagulation Factor Assays with all manual, mechanical, or photo-optical means of clot detection. Follow Reagent manufacturer's recommended procedures for preparation of plasma dilutions using Imidazole Buffered Saline.

### Contents & Determinations

| | |
|---|---|
| Product | IBS Buffer |
| Cat.No. | A0590-125 |
| IBS Buffer | 1x125 mL |

### Preparation
IBS: pH 7.3 - 7.4, liquid
Ready to use.

### Storage and Stability
Unopened reagents are stable until the expiration date shown on the label stored at 2-8°C.

### Precautions
Avoid contact with skin and eyes. Wear suitable protective clothing. Dispose components in compliance with local regulations for infectious material.

### Warranty
This product is warranted to perform in accordance with its labelling and literature. TECO disclaims any implied warranty of merchantability or fitness for any other purpose, and in no event will TECO be liable for any consequential damages arising out of aforesaid express warranty.

Symbols key:

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Expiry date | IVD | In Vitro Diagnostica | | Biological hazard | REF | Catalogue Number | | Consult accompanying documents |
| | Store at 2-8°C | CE | EU conformity | | Manufacturer | LOT | Lot. Number | EC REP | Authorized Representative |

# IBS Buffer

IVD REF *A0590-125*

## Verwendungszweck

Die IBS Pufferlösung (Imidazole Buffered Saline) wird für die Verdünnung von Plasma verwendet werden, wie es z.B. bei der koagulometrischen Bestimmung von Fibrinogen, Einzelfaktoren oder auch Verdünnungsreihen für die Methoden Kalibrierung notwendig ist.

## Inhalte und Bestimmungen

| | |
|---|---|
| Produkt | IBS Puffer |
| Kat.Nr. | A0590-125 |
| IBS Buffer | 1x125 mL |

## Vorbereitung

IBS: pH 7.3 - 7.4, flüssig
Gebrauchsfertig

## Lagerung und Stabilität

Ungeöffnete Reagenzien sind bei Lagerung zwischen 2-8°C bis zum auf dem Etikett angegebenen Verfallsdatum haltbar.

## Vorsichtsmaßnahmen

Haut- und Augenkontakt vermeiden. Angemessene Schutzkleidung tragen. Bestandteile gemäß lokaler Vorschriften für infektiöse Materialien entsorgen.

## Garantie

Es wird garantiert, dass die Wirkungsweise dieses Produktes den Angaben auf der Packung und in der Produktliteratur entspricht. TECO haftet weder für die Verkäuflichkeit oder Eignung dieses Produktes für irgendwelche andere Zwecke noch für irgendwelche Folgeschäden, die sich aus der vorstehenden, expliziten Garantie ergeben.

Erklärung der Symbole:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Verfallsdatum | IVD | In-Vitro Diagnostik | | Biologische Gefahr | REF | Katalog-Nummer | | Begleitpapiere beachten |
| | Bei 2-8°C lagern | CE | EU Konformität | | Hersteller | LOT | Lot. – Nummer | EC REP | Bevollmächtigter |

# TEControl N

CE IVD REF **P6001-010**

## Intended Use
Use as a normal control for following coagulation tests:

**PT, APTT, Thrombintime, Fibrinogen, Antithrombin and D-Dimer**

## Contents
10 x 1mL freeze dried citrate-anticoagulated human plasma

## Preparation
Reconstitute individual vials with **1,0 ml** distilled water. Allow to stand at room temperature, with occasional swirling, for 15 min before use. Be certain all particulate matter is well dissolved.

PT whole blood (TEClot PT-B): Reconstitute individual vials with **1,7 ml** distilled water.

## Storage & Stability
Unopened vials are stable until the expiration date shown on the label stored at 2°-8°C.

Dissolved plasma change analytic levels below 10% if stored as following:

| | | |
|---|---|---|
| -20 °C | 2-8 °C | 20-25 °C |
| 1 month | 8 hours | 4 hours |

Dissolved plasma can be refrozen only one time in aliquots (120-150µL). Stored at -20°C in closed polypropylene tubes, the aliquots must be used within 30 days.

## Precautions
This product contains substance from human origin!
Avoid contact with skin and eyes. Wear suitable protective clothing. Dispose components in compliance with local regulations for infectious material. All components are checked for HIV, HBV and HCV. However products from human blood should be considered as potentially infectious.

## Expected Results
Refer to "Certificate of Analysis".

## Warranty
This product is warranted to perform in accordance with its labelling and literature. TECO disclaims any implied warranty of merchantability or fitness for any other purpose, and in no event will TECO be liable for any consequential damages arising out of aforesaid express warranty.

Symbols key:

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Expiry date | | IVD | In Vitro Diagnostica | | Biological hazard | | REF | Catalogue Number | Consult accompanying documents |
| Store at 2-8°C | | CE | EU conformity | | Manufacturer | | LOT | Lot. Number | EC REP | Authorized Representative |


TECO Medical Instruents Production + Trading GmbH, Dieselstr. 1, 84088 Neufahrn i.NB Germany

# TEControl N

CE IVD REF P6001-010

## Verwendungszweck
Als normale Kontrolle für folgende Gerinnungstests verwenden:

**PT, APTT, Thrombinzeit , Fibrinogen, Antithrombin und D-Dimer**

## Inhalt
10 x 1mL gefriergetrocknetes mit Zitrat versetztes gerinnungshemmendes Humanplasma

## Vorbereitung
Die einzelnen Fläschchen mit 1,0ml destilliertem Wasser anlösen. Fläschchen bei Raumtemperatur bis zur Anwendung unter gelegentlichen Verwirbeln 15 Minuten lang stehen lassen. Stellen Sie sicher, dass alle Partikel gut aufgelöst sind.

Vollblut PT (TEClot PT-B): einzelne Fläschchen mit 1,7ml destilliertem Wasser anlösen.

## Lagerung und Stabilität
Ungeöffnete Fläschchen sind bei Lagerung zwischen 2-8°C zum bis auf dem Etikett angegebenen Verfallsdatum haltbar.

Gelöstes Plasma verändern die analytischen Levels unter 10% wenn wie folgt gelagert:

| | | |
|---|---|---|
| -20 °C | 2-8 °C | 20-25 °C |
| 1 Monat | 8 Stunden | 4 Stunden |

Gelöstes Plasma kann einmalig wiedereingefroren werden. Die Aliquots (120-150µL) sind 30 Tage haltbar, wenn sie in polypropylen Gefäßen bei -20°C aufbewahrt werden.

## Vorsichtsmaßnahmen
Dieses Produkt enthält Substanzen humanen Ursprungs!
Haut- und Augenkontakt vermeiden. Angemessene Schutzkleidung tragen. Abfälle laut lokaler Regelungen für infektiöse Materialien entsorgen. Alle Bestandteile wurden auf HIV, HBV und HCV getestet. Trotzdem müssen Produkte aus menschlichem Blut immer als potentiell infektiös angesehen werden.

## Erwartete Ergebnisse
Lesen Sie das Analysenzertifikat

## Garantie
Es wird garantiert, dass die Wirkungsweise dieses Produkts den Angaben auf der Packung und in der Produktliteratur entspricht. TECO haftet weder für die Verkäuflichkeit oder Eignung dieses Produktes für irgendwelche andere Zwecke noch für irgendwelche Folgeschäden, die sich aus der vorstehenden, expliziten Garantie ergeben.

Erklärung der Symbole:

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Verfallsdatum | IVD | In-Vitro Diagnostik | | Biologische Gefahr | REF | Katalog-Nummer | | Begleitpapiere beachten |
| | Bei 2-8°C lagern | CE | EU Konformität | | Hersteller | LOT | Lot. – Nummer | EC REP | Bevollmächtigter |

# TEControl A

CE IVD REF P6101-010

## Intended Use
Use as an abnormal control for following coagulation tests:

**PT, APTT, Thrombintime, Fibrinogen, Antithrombin and D-Dimer**

## Contents
10 x 1mL freeze dried citrate-anticoagulated human plasma

## Preparation
Reconstitute individual vials with **1,0 ml** distilled water. Allow to stand at room temperature, with occasional swirling, for 15 min before use. Be certain all particulate matter is well dissolved.

PT whole blood (TEClot PT-B): Reconstitute individual vials with **1,7 ml** distilled water.

## Storage & Stability
Unopened vials are stable until the expiration date shown on the label stored at 2°-8°C.

Dissolved plasma change analytic levels below 10% if stored as following:

| -20 °C | 2-8 °C | 20-25 °C |
|---|---|---|
| 1 month | 8 hours | 4 hours |

Dissolved plasma can be refrozen only one time in aliquots (120-150µL). Stored at -20°C in closed polypropylene tubes, the aliquots must be used within 30 days.

## Precautions
This product contains substance from human origin!
Avoid contact with skin and eyes. Wear suitable protective clothing. Dispose components in compliance with local regulations for infectious material. All components are checked for HIV, HBV and HCV. However products from human blood should be considered as potentially infectious.

## Expected Results
Refer to "Certificate of Analysis".

## Warranty
This product is warranted to perform in accordance with its labelling and literature. TECO disclaims any implied warranty of merchantability or fitness for any other purpose, and in no event will TECO be liable for any consequential damages arising out of aforesaid express warranty.

Symbols key:

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Expiry date | IVD | In Vitro Diagnostica | | Biological hazard | REF | Catalogue Number | | Consult accompanying documents |
| Store at 2-8°C | CE | EU conformity | | Manufacturer | LOT | Lot. Number | EC REP | Authorized Representative |

# TEControl A

CE IVD REF P6101-010

## Verwendungszweck
Als abnormale Kontrolle für folgende Gerinnungstests verwenden:

**PT, APTT, Thrombinzeit, Fibrinogen,**
**Antithrombin und D-Dimer**

## Inhalt
10 x 1mL gefriergetrocknetes mit Zitrat versetztes gerinnungshemmendes Humanplasma

## Vorbereitung
Die einzelnen Fläschchen mit 1,0ml destilliertem Wasser anlösen. Fläschchen bei Raumtemperatur bis zur Anwendung unter gelegentlichen Verwirbeln 15 Minuten lang stehen lassen. Stellen Sie sicher, dass alle Partikel gut aufgelöst sind.

Vollblut PT (TEClot PT-B): einzelne Fläschchen mit 1,7ml destilliertem Wasser anlösen.

## Lagerung und Stabilität
Ungeöffnete Fläschchen sind bei Lagerung zwischen 2-8°C zum bis auf dem Etikett angegebenen Verfallsdatum haltbar.

Gelöstes Plasma verändern die analytischen Levels unter 10% wenn wie folgt gelagert:

| | | |
|---|---|---|
| -20 °C | 2-8 °C | 20-25 °C |
| 1 Monat | 8 Stunden | 4 Stunden |

Gelöstes Plasma kann einmalig wiedereingefroren werden. Die Aliquots (120-150µL) sind 30 Tage haltbar, wenn sie in polypropylen Gefäßen bei -20°C aufbewahrt werden.

## Vorsichtsmaßnahmen
Dieses Produkt enthält Substanzen humanen Ursprungs!
Haut- und Augenkontakt vermeiden. Angemessene Schutzkleidung tragen. Abfälle laut lokaler Regelungen für infektiöse Materialien entsorgen. Alle Bestandteile wurden auf HIV, HBV und HCV getestet. Trotzdem müssen Produkte aus menschlichem Blut immer als potentiell infektiös angesehen werden.

## Erwartete Ergebnisse
Lesen Sie das Analysenzertifikat

## Garantie
Es wird garantiert, dass die Wirkungsweise dieses Produkts den Angaben auf der Packung und in der Produktliteratur entspricht. TECO haftet weder für die Verkäuflichkeit oder Eignung dieses Produktes für irgendwelche andere Zwecke noch für irgendwelche Folgeschäden, die sich aus der vorstehenden, expliziten Garantie ergeben.

Erklärung der Symbole:

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Verfallsdatum | IVD | In-Vitro Diagnostik | | Biologische Gefahr | REF | Katalog-Nummer | | Begleitpapiere beachten |
| | Bei 2-8°C lagern | CE | EU Konformität | | Hersteller | LOT | Lot. – Nummer | EC REP | Bevollmächtigter |

ТОВ «ХЕМА» код ЄДРПОУ 36038442
Адреса 03179, м. Київ, вул. Академіка Єфремова, 23
Для кореспонденції: 03179, а/с 49
З питань замовлення продукції: 050-422-62-16, 067-422-62-16
Тел.: +38 (095) 60-99-555 Факс: +38 (044) 422-62-16
e-mail:info@xema.com.ua
www.xema.in.ua

# STATEMENT

We, XEMA LLC, as a manufacturer of in vitro diagnostic medical devices, having a registered office at Akademika Yefremova St. 23, Kyiv, Ukraine assign SRL SANMEDICO having a registered office at A. Corobceanu Street 7A, apt. 9, Chişinău MD-2012, Moldova, as authorized representative in correspondence with legislative requirements of the Republic of Moldova.

We declare that the company mentioned above is authorized to register, notify, renew, or modify the registration of medical devices on the territory of the Republic of Moldova.

This Statement shall come into force on the date of its signing. The duration of this Statement is 3 years from the date of signing.

Date: 06.09.2023

Signature:

Director Xema LLC
Oleksandra Zavaliei

Polmed.de

*Vertretung und Repräsentanz*

# Certificate

## *Of Marketing Authorization of Medical Product*

*within Germany, the member states of the European Union*
*and the other states having a contractual agreement with the European Economic Area*

## Nr. AR/IVD/XEMA LLC/01/2023

Issued on the basis of the Declaration of conformity and registration taking into account account Article 11 of Regulation (EU) 2017/746 (IVDR) on In Vitro Diagnostic, and Medical Device Implementing Act (MPDG)

Ausgestellt auf Grund der Konformitätserklärung und Registrierung unter Berücksichtigung der der Verordnung (EU) 2017/746 (IVDR) über In-vitro-Diagnostika und Medizinprodukterecht-Durchführungsgesetz (MPDG)

| | |
|---|---|
| Manufacturer / Hersteller | **XEMA LLC** **SRN: UA-MF-000032959**<br>UKRAINE, 03179 KYIV<br>Akademika Yefremova St. 23<br>qa@xema.com.ua; www.xema.in.ua |
| Product name / Produkt | **See annex to the Certificate**<br>Siehe Anhang zum Zertifikat |
| Product Classification:<br>Produktklassifizierung | **In Vitro Diagnostic Medical Devices**<br>In-vitro-Diagnostikum (IVD) Medizinprodukte |
| Category:<br>Kategorie | **Common/ Other IVD**<br>Sonstige IVD-Produkte |
| Conformity assessment procedure:<br>Konformitatsbewertungsverfahren: | **EC DECLARATION OF CONFORMITY**<br>**(Annex III, except point 6, Directive 98/79/EC)**<br>**in connection with article 110(3) IVDR**<br>**EU- KONFORMITATSERKLARUNG**<br>(Anhang III, außer Nummer 6, Richtlinie 98/79 / EG)<br>in Verbindung mit Artikel 110 (3) IVDR |
| State Competent Authority:<br>Staatliche Zuständige Behörde | **BfArM** Federal Institute for Drugs and Medical Devices<br>DMIDS (German Medical Device Information and Database System)<br>**BfArM** Das Bundesinstitut für Arzneimittel und Medizinprodukte DMIDS<br>(Deutsches Medizinprodukte-Informations- und Datenbanksystem) |

Date of issue : **2023-03-07**
Das Ausstellungsdatum

Valid to : **2025-05-31**
Gültig bis

Represented in the EC by:

**Polmed.de Beata Rozwadowska**
Fichtenstr. 12A, 90763 Fürth, Germany
email: info@polmed.de
Tel: +49 911 93163967

**SRN: DE-AR-000006947**

Beata Rozwadowska * Polmed.de * Fichtenstr. 12a, 90763 Fürth, Germany

Polmed.de

Valid with the Extract from the database www.dimdi.de (German Medical Device Information and Database System (DMIDS))
Gilt nur mit :Auszug aus der Datenbank www.dimdi.de (Deutsches Medizinprodukte-Informations- und Datenbanksystem (DMIDS))

Polmed.de

*Vertretung und Repräsentanz*

# Annex to the Certificate No.:

Anhang zum Zertifikat Nr.:

## AR/IVD/XEMA LLC/01/2023

The following medical devices can be placed on the market in the Federal Republic of Germany, in the member states of the European Economic Community (EEC) and in the other contract states of the agreement about the European Economic Area.

Die folgenden Medizinprodukte in der Bundesrepublik Deutschland, in den Mitgliedsstaaten der Europäischen Wirtschaftsgemeinschaft (EG) und in den Vertragsstaaten der EG in den Verkehr gebracht werden dürfen.

| # | Nomenclature term<br>Nomenklaturbezeichnung | Catalog No.<br>Katalog-Nr. | Name of device<br>Produktbezeichnung | DMIDS<br>Registration number<br>Registriernummer |
|---|---|---|---|---|
| 1. | ASPERGILLUS | K021 | GalMAg EIA | **DE/CA64/00115824** |
| 2. | HSV IgG | K104 | HSV 1/2 IgG EIA | **DE/CA64/00115826** |
| 3. | HSV IgM | K104M | HSV 1, 2 IgM EIA | **DE/CA64/00115833** |
| 4. | HSV 2 IgG | K104B | HSV 2 IgG EIA | **DE/CA64/00115836** |
| 5. | MYCOPLASMA ANTIBODY ASSAYS | K106 | Mycoplasma IgG EIA | **DE/CA64/00115837** |
| 6. | SYPHILIS ANTIBODY ASSAYS TOTAL | K111 | anti-Treponema pallidum EIA | **DE/CA64/00115839** |
| 7. | SYPHILIS ANTIBODY IGG | K111G | Treponema pallidum IgG EIA | **DE/CA64/00115840** |
| 8. | H. PYLORI ANTIBODY ASSAYS | K119G | Helicobacter pylori IgG EIA | **DE/CA64/00115850** |
| 9. | OTHER OTHER BACTERIOLOGY IMMUNOASSAY | K126 | Ureaplasma IgG EIA | **DE/CA64/00115851** |
| 10. | THYROID PEROXIDASE (INCL. MICROSOMAL) ANTIBODIES | K131 | aTPO EIA | **DE/CA64/00115852** |
| 11. | THYROGLOBULIN AUTOANTIBODIES | K132 | aTG EIA | **DE/CA64/00115853** |
| 12. | MPO ANCA | K133 | aMPO EIA | **DE/CA64/00115854** |
| 13. | TISSUE TRANSGLUTAMINASE ANTIBODIES | K160<br>K161 | anti-TGlu IgG EIA<br>anti-TGlu IgA EIA | **DE/CA64/00115855** |
| 14. | GIARDIA LAMBLIA | K171 | anti-Giardia lamblia EIA | **DE/CA64/00115856** |
| 15. | OTHER PARASITOLOGY | K174 | Ascaris IgG EIA | **DE/CA64/00115857** |
| 16. | ECHINOCOCCUS | K175 | Echinococcus IgG EIA | **DE/CA64/00115858** |
| 17. | DISTOMATOSIS | K176 | Opisthorchis IgG EIA | **DE/CA64/00115859** |
| 18. | GLIADIN ANTIBODIES | K180<br>K181 | Gliadin IgG EIA<br>Gliadin IgA EIA | **DE/CA64/00115860** |
| 19. | IMMUNOGLOBULIN E – TOTAL | K200 | Total IgE EIA | **DE/CA64/00115861** |
| 20. | THYROID STIMULATING HORMONE | K201 | TSH EIA | **DE/CA64/00115863** |
| 21. | LUTEINISING HORMONE | K202 | LH EIA | **DE/CA64/00115864** |
| 22. | FOLLICLE STIMULATING HORMONE | K203 | FSH EIA | **DE/CA64/00115865** |
| 23. | HUMAN GROWTH HORMONE | K204 | GH EIA | **DE/CA64/00115866** |
| 24. | HUMAN CHORIONIC GONADOTROPIN TOTAL | K205 | hCG EIA | **DE/CA64/00115867** |
| 25. | PROLACTIN | K206 | Prolactin EIA | **DE/CA64/00115868** |

The above-mentioned medical products are marked with the CE symbol.
Die oben genannten medizinischen Produkte sind mit dem CE-Zeichen gekennzeichnet.

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|---|---|---|---|---|
| 26. | PROGESTERONE | K207 | Progesterone EIA | **DE/CA64/00115869** |
| 27. | ESTRADIOL | K208 | Estradiol EIA | **DE/CA64/00115870** |
| 28. | TESTOSTERONE (WITH DEHYDRO AND FREE TESTOSTERONE) | K209 | Testosterone EIA | **DE/CA64/00115871** |
| 29. | CORTISOL | K210 | Cortisol EIA | **DE/CA64/00115872** |
| 30. | TRIIODOTHYRONINE | K211 | T3 EIA | **DE/CA64/00115873** |
| 31. | THYROXINE | K212 | T4 EIA | **DE/CA64/00115874** |
| 32. | FREE TRIIODOTHYRONINE | K213 | fT3 EIA | **DE/CA64/00115875** |
| 33. | FREE THYROXINE | K214 | fT4 EIA | **DE/CA64/00115876** |
| 34. | DEHYDRO-EPIANDROSTERONE SULPHATE (INCL. DHEA) | K215 | DHEAS EIA | **DE/CA64/00115877** |
| 35. | 17 OH PROGESTERONE | K217 | 17-OH-progesterone EIA | **DE/CA64/00115878** |
| 36. | ESTRIOL | K218 | free Estriol EIA | **DE/CA64/00115880** |
| 37. | TESTOSTERONE (WITH DEHYDRO AND FREE TESTOSTERONE) | K219 | free Testosterone EIA | **DE/CA64/00115881** |
| 38. | CANCER ANTIGEN 125 | K222 | CA 125 EIA | **DE/CA64/00115882** |
| 39. | CANCER ANTIGEN 19-9 | K223 | CA 19-9 EIA | **DE/CA64/00115883** |
| 40. | CARCINOEMBRYONIC ANTIGEN | K224 | CEA EIA | **DE/CA64/00115884** |
| 41. | ALPHAFETOPROTEIN | K225 | AFP EIA | **DE/CA64/00115885** |
| 42. | CANCER ANTIGEN 15-3 | K226 | CA 15-3 (M12) EIA | **DE/CA64/00115886** |
| 43. | OTHER OTHER TUMOUR MARKERS | K232 | Thyroglobulin EIA | **DE/CA64/00115887** |
| 44. | ß HUMAN CHORIONIC GONADOTROPIN (INCL. SUBUNIT) | K235 | free β-HCG EIA | **DE/CA64/00115888** |
| 45. | CYFRA 21-1 | K236 | CYFRA 21-1 EIA | **DE/CA64/00115889** |
| 46. | SQUAMOUS CELL CARCINOMA ANTIGEN | K237 | SCC (A) EIA | **DE/CA64/00115890** |
| 47. | PREGNANCY ASSOCIATED PLASMA PROTEIN - A (DOWNS) | K238 | PAPP-A EIA | **DE/CA64/00115892** |
| 48. | OTHER OTHER TUMOUR MARKERS | K239 | HE4 EIA | **DE/CA64/00115893** |
| 49. | CANCER ANTIGEN 242 | K243 | CA242 EIA | **DE/CA64/00115894** |
| 50. | OTHER PREGNANCY TESTING HORMONES | K245 | AMH EIA | **DE/CA64/00115896** |

The above-mentioned medical products are marked with the CE symbol.
Die oben genannten medizinischen Produkte sind mit dem CE-Zeichen gekennzeichnet.

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*Vertretung und Repräsentanz*

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Anhang zum Zertifikat Nr.:

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| # | Nomenclature term<br>Nomenklaturbezeichnung | Catalog No.<br>Katalog-Nr. | Name of device<br>Produktbezeichnung | DMIDS<br>Registration number<br>Registriernummer |
|---|---|---|---|---|
| 51. | HUMAN PLACENTAL LACTOGEN HPL | K246 | Placental lactogen EIA | DE/CA64/00115897 |
| 52. | C-REACTIVE PROTEIN | K250 | CRP EIA | DE/CA64/00115898 |
| 53. | C-PEPTIDE | K267C | C-peptide EIA | DE/CA64/00115900 |
| 54. | INSULIN | K267N | Insulin EIA | DE/CA64/00115901 |
| 55. | SEX HORMONE BINDING GLOBULIN | K268 | SHBG EIA | DE/CA64/00115902 |
| 56. | TROPONIN (T + I) | K291 | Troponin I EIA | DE/CA64/00115903 |
| 57. | LYME ANTIBODY IGG | K118G | Borelia burgdorferi IgG EIA | DE/CA64/00115904 |
| 58. | LYME ANTIBODY IGM | K118M | Borelia burgdorferi IgM EIA | DE/CA64/00115905 |
| 59. | EBV ANTIBODIES | K108V<br>K108VM<br>K108N | Epstein-Barr virus VCA IgG EIA<br>Epstein-Barr virus VCA IgM EIA<br>Epstein-Barr virus EBNA IgG EIA | DE/CA64/00115906 |

The above-mentioned medical products are marked with the CE symbol.
Die oben genannten medizinischen Produkte sind mit dem CE-Zeichen gekennzeichnet.

Represented in the EC by:

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email: info@polmed.de
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**SRN: DE-AR-000006947**

Beata Rozwadowska * Polmed.de * Fichtenstr. 12a, 90763 Fürth, Germany

Date: **March 07, 2023**

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ТОВ «УКРМЕДСЕРТ»

# СЕРТИФІКАТ

## про відповідність системи управління якістю

**Зареєстрований у Реєстрі**
**«29» червня 2022 р.**
**№ UA.SM.214-21**
**Дійсний до «03» серпня 2024 р.**
**Перше видання: «04» серпня 2021 р.**

ЦИМ СЕРТИФІКАТОМ ВІДПОВІДНОСТІ ПОСВІДЧУЄТЬСЯ,
ЩО СИСТЕМА УПРАВЛІННЯ ЯКОСТІ СТОСОВНО

**проектування та розроблення, виробництва та дистрибуції медичних виробів для діагностики in vitro**

впроваджена:

**ТОВ «ХЕМА»**

за адресою: **вул. Академіка Єфремова, 23, м. Київ, 03179, Україна**

**відповідає вимогам ISO 13485:2016;**
**ДСТУ EN ISO 13485:2018 (EN ISO 13485:2016, IDT; ISO 13485:2016, IDT).**

Контроль відповідності сертифікованої системи управління якістю вимогам зазначеного стандарту здійснюється шляхом нагляду, періодичність і процедури якого регламентуються процедурами органу з оцінки відповідності.

Сертифікат видано Органом з оцінки відповідності ТОВ «УКРМЕДСЕРТ», акредитованим Національним агентством з акредитації України, атестат від 24.12.2019 № 8О047, адреса: вул. Драгоманова, будинок 1-А, оф. 2, м. Київ, 02059, Україна, тел./факс: +38-067-595-02-30, https://ukrmedcert.org.ua.

**Директор** **І.М. Хотенюк**

**Чинність сертифіката відповідності можна перевірити в Реєстрі на сайті https://ukrmedcert.org.ua та за тел. +38-067-595-02-30**

**Instruction for use**
**A solid-phase enzyme immunoassay kit**
**for the quantitative determination of**
**thyroid microsomal antibodies in human serum or plasma**

# aTPO EIA

Catalogue number REF **K131**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

XEMA LLC
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03179, Kyiv, Ukraine
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tel.:+38 044 294-69-78
E-mail: qa@xema.com.ua
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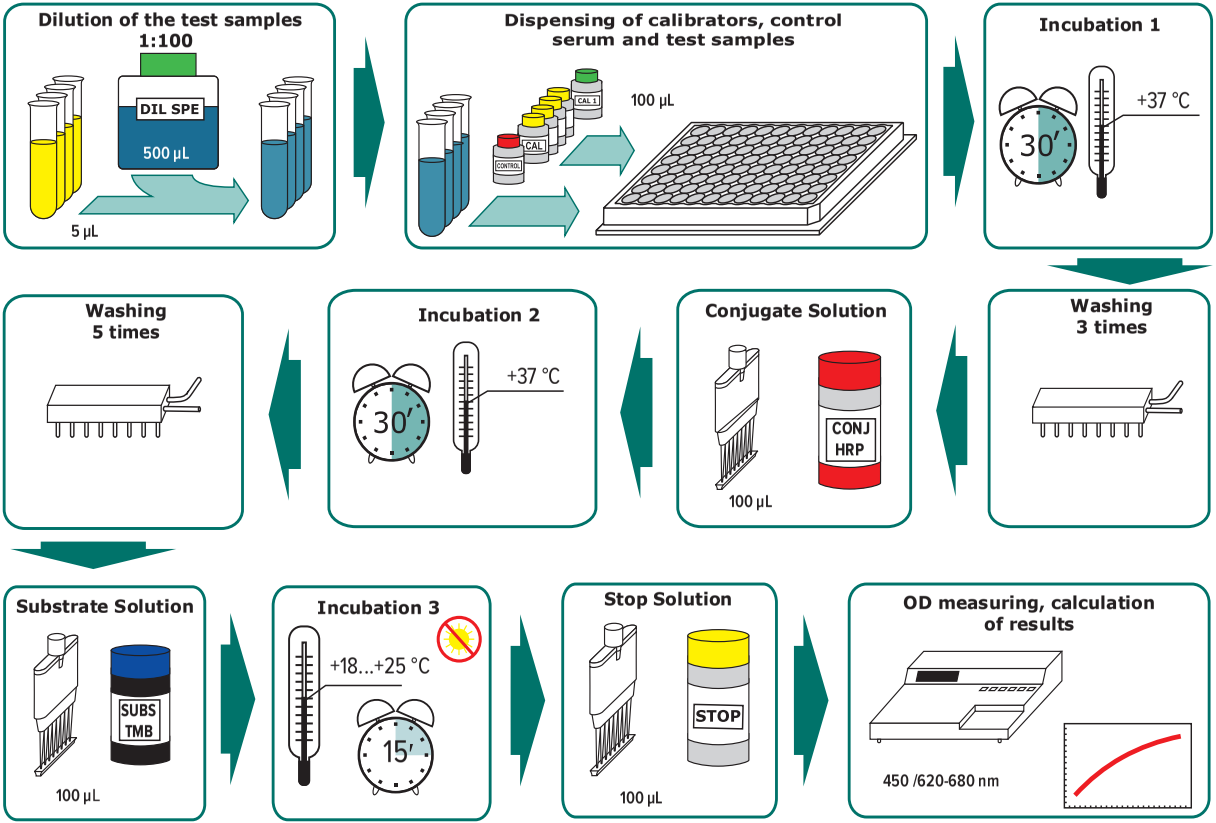
EC REP Authorized Representative in EU:
Polmed.de Beata Rozwadowska
Fichtenstr. 12A, 90763 Fuerth, Germany
tel.:+ 49 911 931 639 67
E-mail: info@polmed.de
www.polmed.de

## ASSAY PROCEDURE




K131

**CONTENT**

# Instruction for use
# A solid-phase enzyme immunoassay kit for the quantitative determination of thyroid microsomal antibodies in human serum or plasma
# aTPO EIA

## 1. INTENDED USE

The aTPO EIA kit is an enzyme immunoassay, intended for the quantitative determination of thyroid microsomal antibodies in human serum or plasma.

The field of application is clinical laboratory diagnostics.

## 2. GENERAL INFORMATION

Anti-TPO antibodies (formerly – thyroid microsomal antibodies) are directed against a target protein – thyroid peroxidase (TPO) – located in the smooth endoplasmic reticulum of thyroid cells. The presence of anti-TPO antibodies in serum is associated with thyroid autoimmune diseases (Graves' disease and Hashimoto's thyroiditis). Anti-TPO antibodies mostly belong to the IgG class.

Low to moderate levels of serum anti-TPO antibodies can be found in some other autoimmune pathology (eg systemic lupus erythematosus or Sjogren syndrom) and, rarely, in apparently healthy subjects (especially elderly women). Anti-TPO antibodies are more sensitive in diagnosis of thyroid autoimmune diseases than anti-thyroglobulin (anti-TG) antibodies. However, in some cases anti-TG positive sera may be negative for anti-TPO. Therefore, combined determination of both types of anti-thyroid antibodies (anti-TPO + anti-TG) provides a more sensitive laboratory diagnostic tool for thyroid autoimmunity.

## 3. PRINCIPLE OF THE TEST

The determination of the anti-TPO antibodies (aTPO) is based on the indirect enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized antigen TPO. Second antibodies – murine monoclonal anti-IgG antibodies conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes three stages of incubation:

- during the first stage specific to antigen TPO antibodies from the specimen are bound by antigens coated onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated murine monoclonal antibodies bind to the antigen-antibody complexes, fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured specific autoantibodies to thyroperoxidase in test specimen.

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of anti-TPO antibodies in the calibration samples.

## 4. KIT COMPONENTS

| Code of component | Symbol | Name | Volume | Qty, pcs. | Description |
|---|---|---|---|---|---|
| P131Z | SORB MTP | **Microplate** | - | 1 | 96-well polystyrene strip microplate coated with antigen TPO; ready to use |
| C131Z | CAL 1 | **Calibrator C1** | 1.1 mL | 1 | Solution based on phosphate buffer (pH 7.2-7.4), free of anti-TPO antibodies, with preservative, ready to use (colourless liquid) |
| C131Z | CAL 2-5 | **Calibrators** | 1.1 mL | 4 | Solutions based on phosphate buffer (pH 7.2-7.4), containing 30; 100; 300 and 1000 IU/mL of anti-TPO antibodies, with preservative, ready to use (red liquids) |
| Q131Z | CONTROL | **Control Serum** | 1.1 mL | 1 | Solution based on human serum, containing of known anti-TPO antibodies content, with preservative, ready to use (colourless liquid) |
| T131Z | CONJ HRP | **Conjugate Solution** | 14 mL | 1 | Solution of murine monocnoclonal antibodies to IgG conjugated to the horseradish peroxidase; ready to use (red liquid) |
| SP131Z | DIL SPE | **EIA Buffer** | 50 mL | 1 | Buffer solution with detergent and preservative, ready to use (blue liquid) |
| R055Z | SUBS TMB | **Substrate Solution** | 14 mL | 1 | Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid) |
| S008Z | BUF WASH 26X | **26x Concentrate Washing Solution** | 30 mL | 1 | Buffer solution with detergent, 26x concentrate (colourless liquid) |
| R050Z | STOP | **Stop Solution** | 14 mL | 1 | 5.0% solution of sulphuric acid; ready to use (colourless liquid) |
| The kit also includes instruction for use, quality control data sheet and plate sealing tape (3 pcs.) | | | | | |

## 5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm or 450\620-680 nm wavelength;
- dry thermostat for +37°C±2°C;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

## 6. WARNING AND PRECAUTIONS

*In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.*

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

**ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.**

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

## 7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

## 8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The aTPO EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The aTPO EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- EIA Buffer, Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

## 9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing solution preparation

Add the contents of the 30 mL washing solution concentrate vial to 750 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

| Quantity of strips | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Volume of the washing solution concentrate, mL | 2.5 | 5 | 7.5 | 10 | 12.5 | 15 | 17.5 | 20 | 22.5 | 25 | 27.5 | 30 |
| Volume of water, mL | 62.5 | 125 | 187.5 | 250 | 312.5 | 375 | 437.5 | 500 | 562.5 | 625 | 687.5 | 750 |

9.4. Samples preparation

Dilute samples using EIA buffer 101 fold (for example, add to the vial 5 µL of the test sample + 500 µL EIA buffer).

If suggested analyte concentration in the sample exceeds the 1000 IU/mL, additionally dilute this sample accordingly, using EIA buffer. Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

*NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of biological fluids.*

***Do not dilute Control Serum and Calibrators!***

## 10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 Dilute the test samples as described in 9.4.

10.3 Dispense **100 µL of Calibrators and Control Serum as well as 100 µL of diluted test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

*NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.*

***Scheme of introduction of samples***

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | CAL1 | CAL1 | SAMP3 | SAMP3 | SAMP11 | SAMP11 | | | | | | |
| B | CAL2 | CAL2 | SAMP4 | SAMP4 | SAMP12 | SAMP12 | | | | | | |
| C | CAL3 | CAL3 | SAMP5 | SAMP5 | SAMP13 | SAMP13 | | | | | | |
| D | CAL4 | CAL4 | SAMP6 | SAMP6 | SAMP14 | SAMP14 | | | | | | |
| E | CAL5 | CAL5 | SAMP7 | SAMP7 | SAMP15 | SAMP15 | | | | | | |
| F | Q | Q | SAMP8 | SAMP8 | | | | | | | | |
| G | SAMP1 | SAMP1 | SAMP9 | SAMP9 | | | | | | | | |
| H | SAMP2 | SAMP2 | SAMP10 | SAMP10 | | | | | | | | |

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 3 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6 Add **100 µL of Conjugate Solution** to all wells.

10.7 Cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.8 At the end of the incubation period, aspirate and wash each well 5 times as described in 10.5.

10.9 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.10 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.11 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution. Set photometer blank on CAL1.

10.12 Plot a calibration curve in linear coordinates: (x) is the concentration of aTPO IU/mL in the calibrators, (y) – OD versus aTPO concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.13 Determine the corresponding concentration of aTPO in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

10.14 The aTPO EIA kit can be used for screening. For this purpose, it is necessary to add 100 µL of Calibrator CAL1 to the wells of the microplate in duplicates, and 100 µL of Calibrator CAL2 30 IU/mL to other wells in duplicates, to the rest wells - 100 µL of diluted tested samples. Compare the value of OD of each tested serum (plasma) sample with the OD of calibrator CAL2 30 IU/ml (IU/ml) (ODC). If the OD value of the test sample is higher than the ODC value (+10%), then the result should be considered as POSITIVE (more than 30 IU/ml aTPO). If the OD value of the tested sample is lower than the ODC value (-10%), then the result should be considered as NEGATIVE. If the OD value of the tested sample is within ± 10%, then this result should be considered EQUIVOCAL.

## 11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

## 12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for aTPO. Based on data obtained by XEMA, the following normal range is recommended (see below).

*NOTE: values of aTPO concentrations in the tested samples that are below the LoD (2.5 IU/mL) and also exceed the value of the upper calibrator (1000 IU/mL) should be provided in the following form: «the aTPO concentration of tested sample X is «lower than 2.5 IU/mL» or «higher than 1000 IU/mL».*

| Sex, age | Units, IU/mL | |
|---|---|---|
| | Lower limit | Upper limit |
| Males | - | 30 |
| Females | - | 30 |
| Females >50 yrs | - | 50 |

## 13. PERFORMANCE CHARACTERISTICS

### 13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

*Repeatability (Intra assay repeatability)* was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

| Sample | Concentration, IU/mL | CV, % |
|---|---|---|
| 1 | 322.4 | 6.74 |
| 2 | 175.2 | 5.62 |

*Reproducibility (Inter assay reproducibility)* was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

| Sample | Concentration, IU/mL | CV, % |
|---|---|---|
| 1 | 341.6 | 7.15 |
| 2 | 181.7 | 4.48 |

*Reproducibility between lots* was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

| Sample | Concentration1, IU/mL | Concentration2, IU/mL | Concentration3, IU/mL | CV, % |
|---|---|---|---|---|
| 1 | 352.6 | 358.4 | 360.1 | 2.1 |
| 2 | 182.6 | 198.7 | 200.4 | 6.1 |

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known aTPO concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 30-300 IU/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest aTPO concentration in the serum or plasma sample that is detected by the aTPO EIA kit is no lower than 2.5 IU/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for aTPO EIA kit is 20 IU/mL.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

## 14. REFERENCES

1. Amino N., Mosi H., Iwatani W., Tanizawa O., Kawashima M., Tsuge I., Ibiragi K., Kumahara Y., Miyai K. – High prevalence of transient postpartum thyrotoxicosis and hypothyroidism. New Engl.J.Med., 1982, 306:84.
2. Bastenie P., Neve P., Bonnyns M., Van Haelts L., Chailly M. – Clinical and pathological significance of atrophic thyroiditis. Lancet, 1967, 1:915.
3. Bonnyns M., Van Haelts L., Bastenie P. – Asymptomatic atrophic thyroiditis. Horm. Res. 1982, 16:338.
4. Buchanan W., Alexander W., Crooks J., Koutras D., Wayne E., Anderson J.R., Goudie R. – Association of thyrotoxicosis and autoimmune thyroiditis. Brit.Med. J., 1961, 1:843.
5. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
6. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
7. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

## SAMPLES IDENTIFICATION PLAN

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | | | | | | | | | | | | |
| **B** | | | | | | | | | | | | |
| **C** | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | |
| **F** | | | | | | | | | | | | |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |

LOT _____ DATE _____

## SAMPLES IDENTIFICATION PLAN

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | | | | | | | | | | | | |
| **B** | | | | | | | | | | | | |
| **C** | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | |
| **F** | | | | | | | | | | | | |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |

LOT _____ DATE _____

| | |
|---|---|
|  | Manufacturer |
|  | *In vitro* diagnistic medical device |
|  | Catalogue number |
|  | Use-by date |
|  | Batch code |
|  | Temperature limit |
|  | Contains sufficient for <n> tests |
|  | Caution |
|  | Consult instructions for use |
|  | Conformity Marking with technical regulations in Ukraine |
|  | Authorized representative in the European Community/European Union |
|  | CE Conformity Marking |

**For any issues related to operation of the kit and technical support, please contact by telefon number**

**+38 044 294-69-78**

**or write to:**

**qa@xema.com.ua**

XEMA LLC
Akademika Yefremova St. 23
03179, Kyiv, Ukraine
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tel.:+38 044 294-69-78
E-mail: qa@xema.com.ua
www.xema.com.ua

**Instruction for use**
**A solid-phase enzyme immunoassay kit for the quantitative determination of autoantibodies to thyroglobulin in human serum or plasma**

# aTG EIA

Catalogue number REF **K132**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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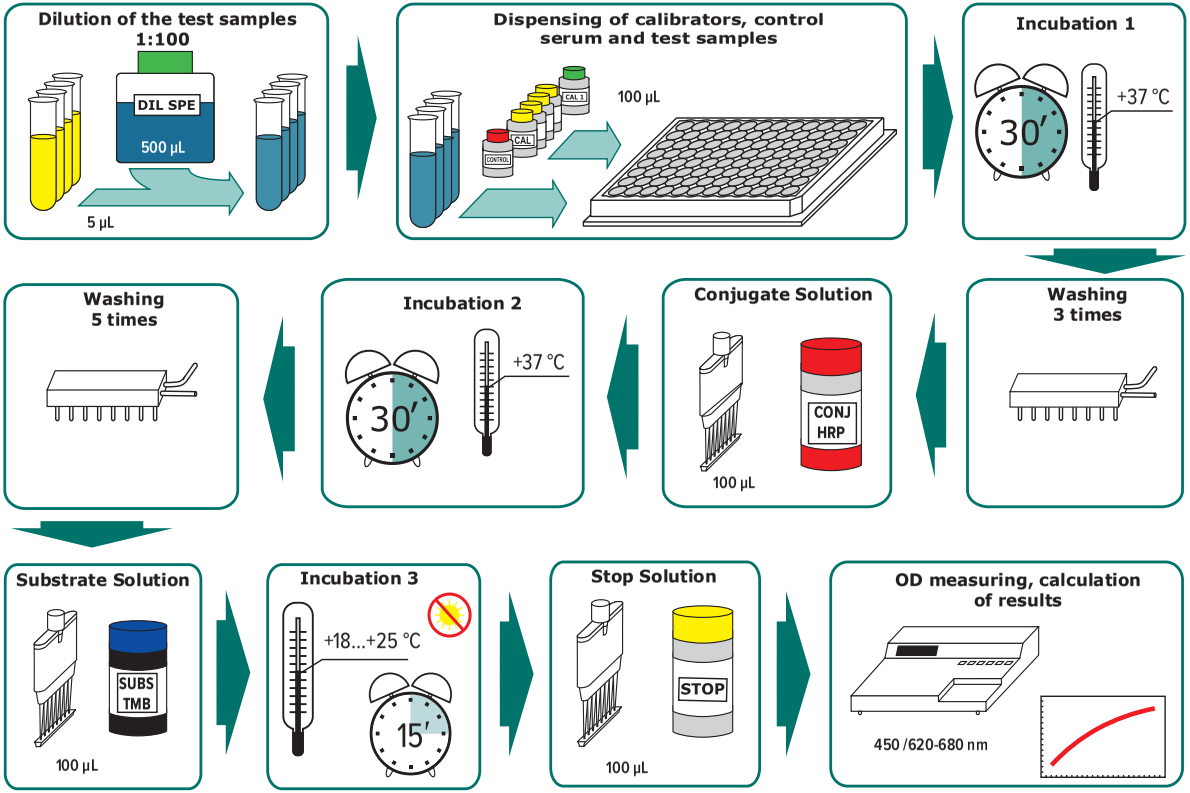
tel.:+ 49 911 931 639 67
E-mail: info@polmed.de
www.polmed.de

## ASSAY PROCEDURE




K132

**CONTENT**

# Instruction for use
# A solid-phase enzyme immunoassay kit for the quantitative determination of autoantibodies to thyroglobulin in human serum or plasma
# aTG EIA

## 1. INTENDED USE

The aTG EIA kit is an enzyme immunoassay, intended for the quantitative determination of autoantibodies to thyroglobulin in human serum or plasma.

The field of application is clinical laboratory diagnostics.

## 2. GENERAL INFORMATION

Thyroglobulin (TG) is a well known target for autoantibodies occurring in thyroid autoimmunity (Graves' disease and Hashimoto's thyroiditis). Anti-TG antibodies mostly belong to the IgG class. Low to moderate levels of anti-TG antibodies can be found in sera of other autoimmune patients (eg systemic lupus erythematosus or Sjogren syndrome).

In some cases anti-TG positive sera may show negativity for other type of anti-thyroid antibodies – anti-TPO. Therefore, combined determination of both types of anti-thyroid antibodies (anti-TPO + anti-TG) provides most sensitive laboratory diagnostic tool for thyroid autoimmunity. Separately from autoimmunity, anti-TG antibodies may develop in patients suffering from thyroid cancer. High level of anti-TG in such patients may interfere with correct determination of serum thyroglobulin which serves as tumour marker for therapy control in this group of patients.

## 3. PRINCIPLE OF THE TEST

The determination of the anti-TG antibodies (aTG) is based on the indirect enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized antigen Thyroglobulin. Second antibodies – murine monoclonal anti-IgG antibodies conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes three stages of incubation:

- during the first stage specific to antigen anti-TG antibodies from the specimen are bound by antigens coated onto the microwell surface;
- during the second stage horseradish peroxidase-conjugated murine monoclonal antibodies bind to the antigen-antibody complexes, fixed in the formed at the previous stage complexes;
- during the third stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured specific autoantibodies to thyroglobulin in test specimen.

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of anti-TG antibodies in the calibration samples.

## 4. KIT COMPONENTS

| Code of component | Symbol | Name | Volume | Qty, pcs. | Description |
|---|---|---|---|---|---|
| P132Z | SORB MTP | **Microplate** | - | 1 | 96-well polystyrene strip microplate coated with antigen Thyroglobulin; ready to use |
| C132Z | CAL 1 | **Calibrator C1** | 1.1 mL | 1 | Solution based on phosphate buffer (pH 7.2-7.4), free of anti-TG antibodies, with preservative, ready to use (colourless liquid) |
| C132Z | CAL 2-5 | **Calibrators** | 1.1 mL | 4 | Solutions based on phosphate buffer (pH 7.2-7.4), containing 100; 300; 1000 and 3000 IU/mL of anti-TG antibodies, with preservative, ready to use (blue liquids) |
| Q132Z | CONTROL | **Control Serum** | 1.1 mL | 1 | Solution based on human serum, containing of known anti-TG antibodies content, with preservative, ready to use (colourless liquid) |
| T132Z | CONJ HRP | **Conjugate Solution** | 14 mL | 1 | Solution of murine monocnoclonal antibodies to IgG conjugated to the horseradish peroxidase; ready to use (magenta liquid) |
| S011Z3 | DIL | **EIA Buffer** | 50 mL | 1 | Buffer solution with detergent and preservative, ready to use (blue liquid) |
| R055Z | SUBS TMB | **Substrate Solution** | 14 mL | 1 | Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid) |
| S008Z | BUF WASH 26X | **26x Concentrate Washing Solution** | 22 mL | 2 | Buffer solution with detergent, 26x concentrate (colourless liquid) |
| R050Z | STOP | **Stop Solution** | 14 mL | 1 | 5.0% solution of sulphuric acid; ready to use (colourless liquid) |
| The kit also includes instruction for use, quality control data sheet and plate sealing tape (3 pcs.) | | | | | |

## 5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm or 450\620-680 nm wavelength;
– dry thermostat for +37°C±1°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

## 6. WARNING AND PRECAUTIONS

*In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.*

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

**ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.**

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

## 7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

## 8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The aTG EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The aTG EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- EIA Buffer, Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

## 9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing solution preparation

Add the contents of the 22 mL washing solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

| Quantity of strips | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Volume of the washing solution concentrate, mL | 1.8 | 3.6 | 5.4 | 7.2 | 9 | 10.8 | 12.6 | 14.4 | 16.2 | 18 | 19.8 | 22 |
| Volume of water, mL | 45 | 90 | 135 | 180 | 225 | 270 | 315 | 360 | 405 | 450 | 495 | 550 |

9.4. Samples preparation

Dilute samples using EIA buffer 101 fold (for example, add to the vial 5 µL of the test sample + 500 µL EIA buffer).

If suggested analyte concentration in the sample exceeds the 3000 IU/mL, additionally dilute this sample accordingly, using EIA buffer. Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

*NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of biological fluids.*

***Do not dilute Control Serum and Calibrators!***

## 10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-5) and 2 wells for Control Serum (Q)).

10.2 Dilute the test samples as described in 9.4.

10.3 Dispense **100 µL of Calibrators and Control Serum as well as 100 µL of diluted test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

*NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.*

***Scheme of introduction of samples***

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | CAL1 | CAL1 | SAMP3 | SAMP3 | SAMP11 | SAMP11 | | | | | | |
| B | CAL2 | CAL2 | SAMP4 | SAMP4 | SAMP12 | SAMP12 | | | | | | |
| C | CAL3 | CAL3 | SAMP5 | SAMP5 | SAMP13 | SAMP13 | | | | | | |
| D | CAL4 | CAL4 | SAMP6 | SAMP6 | SAMP14 | SAMP14 | | | | | | |
| E | CAL5 | CAL5 | SAMP7 | SAMP7 | SAMP15 | SAMP15 | | | | | | |
| F | Q | Q | SAMP8 | SAMP8 | | | | | | | | |
| G | SAMP1 | SAMP1 | SAMP9 | SAMP9 | | | | | | | | |
| H | SAMP2 | SAMP2 | SAMP10 | SAMP10 | | | | | | | | |

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 3 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6 Add **100 µL of Conjugate Solution** to all wells.

10.7 Cover strips with a plate sealing tape and incubate for **30 minutes at +37°C**.

10.8 At the end of the incubation period, aspirate and wash each well 5 times as described in 10.5.

10.9 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18…+25°C) for 15 minutes**.

10.10 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.11 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the stop solution. Set photometer blank on CAL1.

10.12 Plot a calibration curve in linear coordinates: (x) is the concentration of aTG IU/mL in the calibrators, (y) – OD versus aTG concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.13 Determine the corresponding concentration of aTG in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

## 11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

## 12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for aTG. Based on data obtained by XEMA, the following normal range is recommended (see below).

*NOTE: values of aTG concentrations in the tested samples that are below the LoD (5.0 IU/mL) and also exceed the value of the upper calibrator (3000 IU/mL) should be provided in the following form: «the aTG concentration of tested sample X is «lower than 5.0 IU/mL» or «higher than 3000 IU/mL».*

| Sex, age | Units, IU/mL | |
|---|---|---|
| | Lower limit | Upper limit |
| Males | - | 100 |
| Females | - | 100 |
| Females >50 yrs | - | 150 |

## 13. PERFORMANCE CHARACTERISTICS

### 13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

*Repeatability (Intra assay repeatability)* was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

| Sample | Concentration, IU/mL | CV, % |
|---|---|---|
| 1 | 1256.9 | 2.46 |
| 2 | 110.7 | 5.39 |

*Reproducibility (Inter assay reproducibility)* was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

| Sample | Concentration, IU/mL | CV, % |
|---|---|---|
| 1 | 1264.5 | 4.33 |
| 2 | 107.9 | 6.43 |

*Reproducibility between lots* was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

| Sample | Concentration1, IU/mL | Concentration2, IU/mL | Concentration3, IU/mL | CV, % |
|---|---|---|---|---|
| 121 | 1270.5 | 1262.8 | 1276.6 | 0.54 |
| 433 | 109.4 | 114.5 | 118.5 | 4.00 |

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known aTG concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 100-3000 IU/mL ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest aTG concentration in the serum or plasma sample that is detected by the aTG EIA kit is no lower than 5 IU/mL.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for aTG EIA kit is 100 IU/mL.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

## 14. REFERENCES

1. U Feldt-Rasmussen – Analytical and clinical performance goals for testing autoantibodies to thyroperoxidase, thyroglobulin, and thyrotropin receptor. Clin. Chem., Jan 1996; 42: 160 – 163.
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4. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
5. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
6. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

## SAMPLES IDENTIFICATION PLAN

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | | | | | | | | | | | | |
| **B** | | | | | | | | | | | | |
| **C** | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | |
| **F** | | | | | | | | | | | | |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |

LOT _____ DATE _____

## SAMPLES IDENTIFICATION PLAN

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | | | | | | | | | | | | |
| **B** | | | | | | | | | | | | |
| **C** | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | |
| **F** | | | | | | | | | | | | |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |

LOT _____ DATE _____

| | |
|---|---|
|  | Manufacturer |
|  | *In vitro* diagnistic medical device |
|  | Catalogue number |
|  | Use-by date |
|  | Batch code |
|  | Temperature limit |
|  | Contains sufficient for <n> tests |
|  | Caution |
|  | Consult instructions for use |
|  | Conformity Marking with technical regulations in Ukraine |
|  | Authorized representative in the European Community/European Union |
|  | CE Conformity Marking |

**For any issues related to operation of the kit and technical support, please contact by telefon number**

**+38 044 294-69-78**

**or write to:**

**qa@xema.com.ua**

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**Instruction for use**
**A solid-phase enzyme immunoassay kit for the quantitative determination of thyroid stimulating hormone in human serum or plasma**

# TSH EIA

Catalogue number REF **K201**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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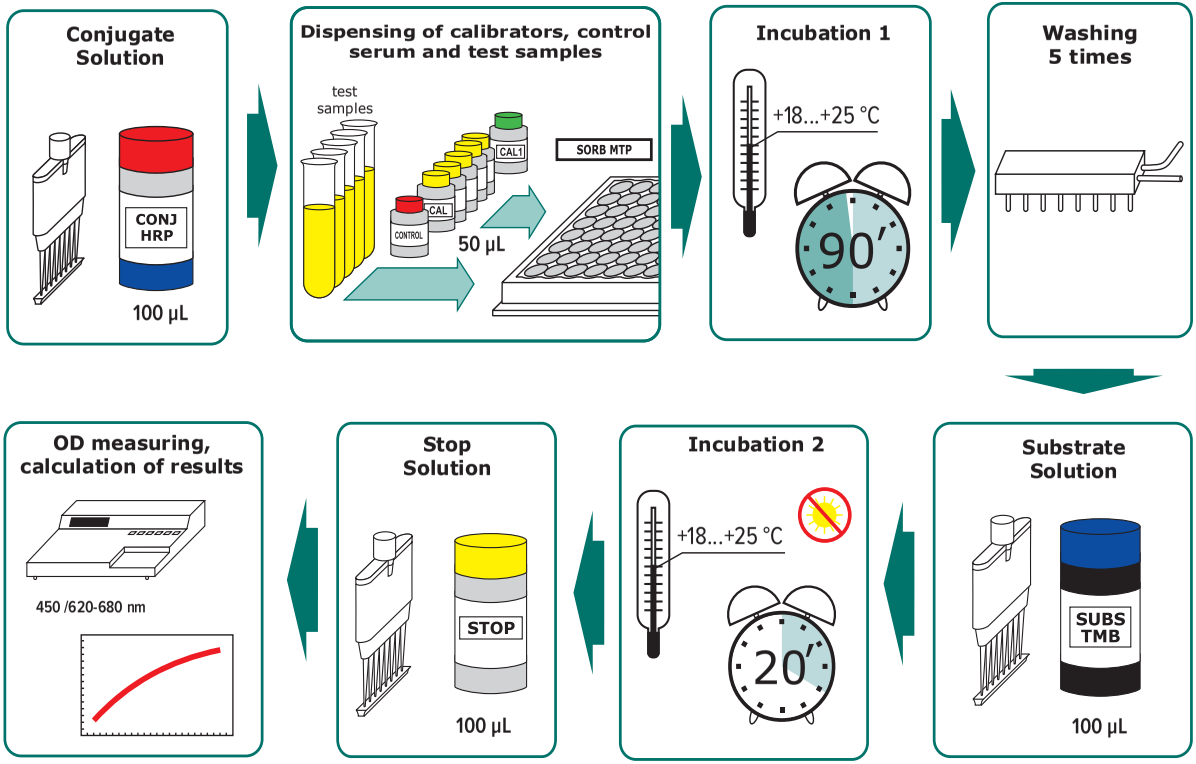
## ASSAY PROCEDURE




## K201

**CONTENT**

# Instruction for use
# A solid-phase enzyme immunoassay kit for the quantitative determination of thyroid stimulating hormone in human serum or plasma
# TSH EIA

## 1. INTENDED USE

The TSH EIA kit is an enzyme immunoassay, intended for the quantitative determination of thyroid stimulating hormone in human serum or plasma.

The field of application is clinical laboratory diagnostics.

## 2. GENERAL INFORMATION

Thyroid stimulating hormone (TSH) is a glycoprotein with molecular weight ca.30 kDa which is secreted by hypophysis. A molecule of TSH consists of two noncovalently bound subunits: α and β. β-subunit determines biological activity and immunological specificity of TSH.

TSH stimulates thyroid gland to secrete thyroid hormones. When the concentration of these hormones in blood serum increases secretion of TSH is inhibited; on the contrary, when the level of thyroid hormones decreases, in the pituitary gland, the release of TSH increases, and therefore the production and release increases thyroid hormones.TSH secretion is subject to circadian rhythms with highest levels seen early in the morning (6 a.m.). Changes of TSH blood level during a day are not significant; nevertheless, if the results do not correspond with clinical status and other laboratory data, it is recommended to take and test another blood sample.

Determination of TSH level in serum is recommended in the following states and conditions:

1) diagnostics of dysfunction of the thyroid gland;

2) hypothyroidism (TSH level is increased. The diagnosis is confirmed by low concentrations of total and free T4 and T3. In mild subclinical forms when T4 and T3 levels are within normal ranges, determination of TSH concentration is critical);

3) hyperthyroidism (synthesis and secretion of TSH are inhibited); monitoring of replacement therapy;

4) screening for congenital hypothyroidism (on the fifth day of life, the level is determined TSH in a blood spot on filter paper or in blood serum). TSH level elevated at birth (up to 35 mIU/L), but after a few days it decreases to basal (both in boys and in girls).

Serum TSH level is elevated during pregnancy, after physical stress, in individuals with lowered blood pressure and lowered temperature. Secretion of TSH is inhibited by Cortisol and Growth hormone. Low TSH levels are often seen in elderly people, in patients with chronic renal insufficiency, liver cirrhosis, in retardation of sexual development, in secondary amenorrhea, Cushing syndrome, acromegaly.

## 3. PRINCIPLE OF THE TEST

The determination of TSH is based on the two-site sandwich enzyme immunoassay principle. On the inner surface of the microplate wells are immobilized specific murine monoclonal antibodies to β-chain of human TSH. Second antibodies – Fab 2 fragment of murine monoclonal antibodies to human TSH conjugated to the horseradish peroxidase is used as enzyme conjugate.

The analysis procedure includes two stages of incubation:

- during the first stage TSH from the specimen is captured by the antibodies coated onto the microwell surface, as well as horseradish peroxidase-conjugated monoclonal antibodies bind to free epitopes of immobilized TSH;
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is directly related to the quantity of the measured TSH in the serum specimen (plasma). The concentration is determined according to the calibration graph of the dependence of the optical density on the content of TSH in the calibration samples.

## 4. KIT COMPONENTS

| Code of component | Symbol | Name | Volume | Qty, pcs. | Description |
|---|---|---|---|---|---|
| P201Z | SORB MTP | **Microplate** | - | 1 | 96-well polystyrene strip microplate coated with murine monoclonal antibodies to β-chain of human TSH; ready to use |
| C201Z | CAL 1 | **Calibrator C1** | 2 mL | 1 | Solution based on phosphate buffer (pH 7.2-7.4), free of human TSH, with preservative, ready to use (yellow liquid) |
| C201Z | CAL 2-6 | **Calibrators** | 0.8 mL | 5 | Solution based on phosphate buffer (pH 7.2-7.4), containing 0,2; 1; 5; 10 and 20 mIU/L of human TSH, with preservative, ready to use (red liquids) |
| Q201Z | CONTROL | **Control Serum** | 0.8 mL | 1 | Solution based on human serum, containing of known human TSH content, with preservative, ready to use (colourless liquid) |
| T201Z | CONJ HRP | **Conjugate Solution** | 14 mL | 1 | Solution of Fab 2 fragment of murine monoclonal antibodies to human TSH conjugated to the horseradish peroxidase; ready to use (blue liquid) |
| R055Z | SUBS TMB | **Substrate Solution** | 14 mL | 1 | Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid) |
| S008Z | BUF WASH 26X | **26x Concentrate Washing Solution** | 22 mL | 1 | Buffer solution with detergent, 26x concentrate (colourless liquid) |
| R050Z | STOP | **Stop Solution** | 14 mL | 1 | 5.0% solution of sulphuric acid; ready to use (colourless liquid) |
| The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.) | | | | | |

## 5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength or 450\620-680 nm;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

## 6. WARNING AND PRECAUTIONS

*In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.*

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

**ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.**

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

## 7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

## 8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The TSH EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The TSH EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months.

  *NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed.*
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

  Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

## 9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

| Quantity of strips | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Volume of the Washing Solution concentrate, mL | 1.8 | 3.6 | 5.4 | 7.2 | 9 | 10.8 | 12.6 | 14.4 | 16.2 | 18 | 19.8 | 22 |
| Volume of water, mL | 45 | 90 | 135 | 180 | 225 | 270 | 315 | 360 | 405 | 450 | 495 | 550 |

9.4. Samples preparation

If suggested analyte concentration in the sample exceeds the 20 mIU/L, additionally dilute this sample accordingly, using (Calibrator C1). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

*NOTE: in order to obtain reliable results, we recommend to use several successive dilutions of the blood serum (plasma) sample*

***Do not dilute Control Serum and Calibrators!***

## 10. ПРОВЕДЕННЯ АНАЛІЗУ

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 14 wells for Calibrators and Control Serum (2 wells for each Calibrator (CAL 1-6) and 2 wells for Control Serum (Q)).

10.2 If necessary, dilute the test samples as described in 9.4.

10.3 Dispense **100 µL of Conjugate Solution** to all wells.

10.4 Dispense **50 µL of Calibrators and Control Serum as well as 50 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

*NOTE: during performing several independent series of tests, Calibrators, and Control Serum should be used each time.*

***Scheme of introduction of samples***

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | CAL1 | CAL1 | SAMP2 | SAMP2 | SAMP10 | SAMP10 | | | | | | |
| B | CAL2 | CAL2 | SAMP3 | SAMP3 | SAMP11 | SAMP11 | | | | | | |
| C | CAL3 | CAL3 | SAMP4 | SAMP4 | SAMP12 | SAMP12 | | | | | | |
| D | CAL4 | CAL4 | SAMP5 | SAMP5 | | | | | | | | |
| E | CAL5 | CAL5 | SAMP6 | SAMP6 | | | | | | | | |
| F | CAL6 | CAL6 | SAMP7 | SAMP7 | | | | | | | | |
| G | Q | Q | SAMP8 | SAMP8 | | | | | | | | |
| H | SAMP1 | SAMP1 | SAMP9 | SAMP9 | | | | | | | | |

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **90 minutes at room temperature (+18...+25°C)**.

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the wash solution volume can be increased to 350 µL.

10.7 Add **100 µL of Substrate Solution** to all wells. The introduction of the Substrate Solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 20 minutes**.

10.8 Add **100 µL of Stop Solution** to all wells in the same order as the Substrate Solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.9 Read the optical density (OD) of the wells at 450nm and reference light filters 620–680 nm using a microplate photometer within 5 minutes of adding the Stop Solution. Set photometer blank on CAL1.

10.10 Plot a calibration curve in linear coordinates: (x) is the TSH concentration in the calibrators mIU/L, (y) – OD versus TSH concentration (OD 450 nm / 620–680 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

10.11 Determine the corresponding concentration of TSH in tested samples from the calibration curve. In the case of preliminary dilution of the test sample (see 9.4), the obtained result should be multiplied by the dilution factor.

## 11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 0.15, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

## 12. EXPECTED VALUES

Therapeutical consequences should not be based on the results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for TSH. Based on data obtained by XEMA, the following normal range is recommended (see below).

*NOTE: values of TSH concentrations in the tested samples that are below the LoD (0.04 mIU/L) and also exceed the value of the upper Calibrator (20 mIU/L) should be provided in the following form : «the TSH concentration of tested sample X is «lower than 0.04 mIU/L» or «higher than 20 mIU/L»*.

| Sex, age | Units, mIU/L | |
|---|---|---|
| | Lower limit | Upper limit |
| Healthy donors | 0.3 | 4.0 |

## 13. PERFORMANCE CHARACTERISTICS

### 13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

*Repeatability (Intra assay repeatability)* was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

| Sample | Concentration, mIU/L | CV, % |
|---|---|---|
| 1 | 2.12 | 7.2 |
| 2 | 3.64 | 3.8 |

*Reproducibility (Inter assay reproducibility)* was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

| Sample | Concentration, mIU/L | CV, % |
|---|---|---|
| 1 | 2.27 | 12.0 |
| 2 | 3.87 | 6.4 |

*Reproducibility between lots* was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

| Sample | Concentration1, mIU/L | Concentration2, mIU/L | Concentration3, mIU/L | CV, % |
|---|---|---|---|---|
| 1 | 2.32 | 2.02 | 1.81 | 9.9 |
| 2 | 3.71 | 3.56 | 3.32 | 5.6 |

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known TSH concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 0.2-10 mIU/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest TSH concentration in the serum or plasma sample that is detected by the TSH EIA kit is no lower than 0.04 mIU/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for TSH EIA kit is 0,15 mIU/L.

13.1.5 Hook Effect

Hook effect is absent for all samples up to reasonably foreseen concentrations 20 mIU/L.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of TSH with other analytes is shown in the table:

| Analyte | Cross-reactivity, % |
|---|---|
| HCG | < 0.1 |
| LH | < 0.1 |
| FSH | < 0.1 |

## 14. REFERENCES

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7. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
8. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

## SAMPLES IDENTIFICATION PLAN

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | | | | | | | | | | | | |
| **B** | | | | | | | | | | | | |
| **C** | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | |
| **F** | | | | | | | | | | | | |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |

LOT _____ DATE _____

## SAMPLES IDENTIFICATION PLAN

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** | | | | | | | | | | | | |
| **B** | | | | | | | | | | | | |
| **C** | | | | | | | | | | | | |
| **D** | | | | | | | | | | | | |
| **E** | | | | | | | | | | | | |
| **F** | | | | | | | | | | | | |
| **G** | | | | | | | | | | | | |
| **H** | | | | | | | | | | | | |

LOT _____ DATE _____

| | |
|---|---|
|  | Manufacturer |
|  | *In vitro* diagnistic medical device |
|  | Catalogue number |
|  | Use-by date |
|  | Batch code |
|  | Temperature limit |
|  | Contains sufficient for <n> tests |
|  | Caution |
|  | Consult instructions for use |
|  | Conformity Marking with technical regulations in Ukraine |
|  | Authorized representative in the European Community/European Union |
|  | CE Conformity Marking |

**For any issues related to operation of the kit and technical support, please contact by telefon number**

**+38 044 294-69-78**

**or write to:**

**qa@xema.com.ua**

XEMA LLC
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www.xema.in.ua

**Instruction for use**
**A solid-phase enzyme immunoassay kit**
**for the quantitative determination of**
**triiodothyronine in human serum or plasma**

# T3 EIA

Catalogue number REF **K211**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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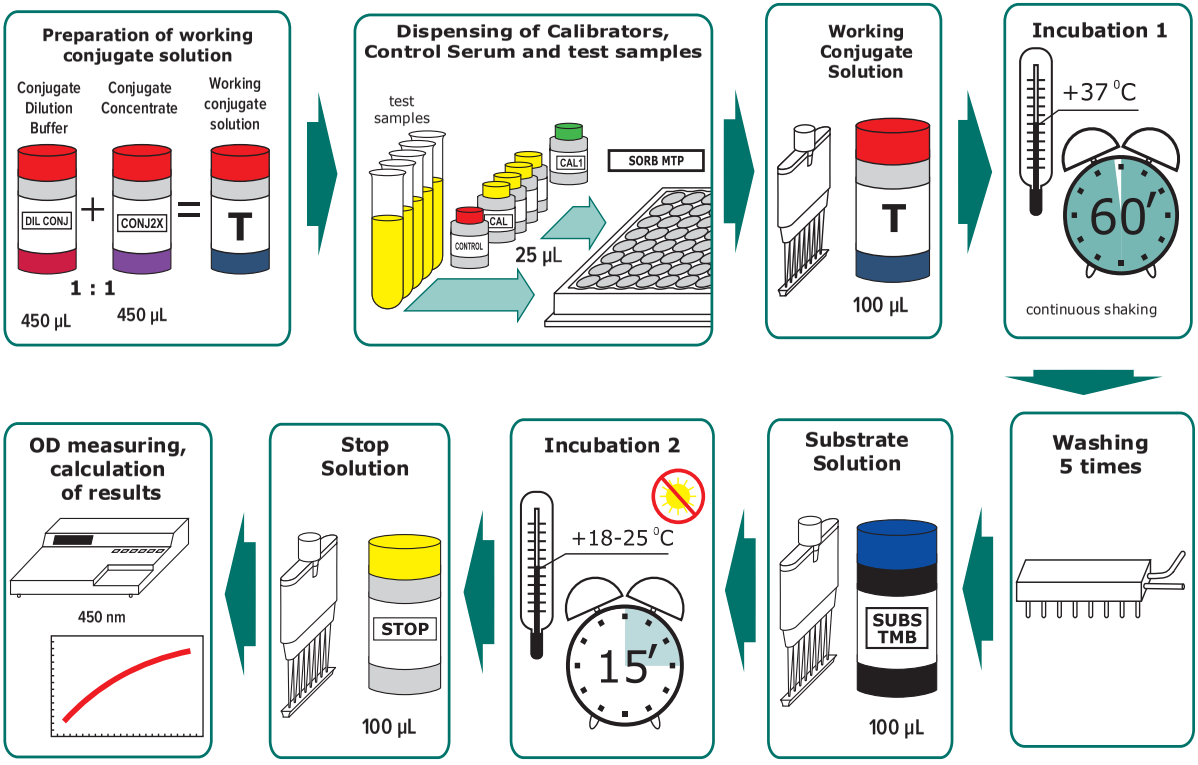
## ASSAY PROCEDURE




K211

**CONTENT**

# Instruction for use
# A solid-phase enzyme immunoassay kit for the quantitative determination of triiodothyronine in human serum or plasma
# T3 EIA

## 1. INTENDED USE

The T3 EIA kit is an enzyme immunoassay, intended for the quantitative determination of triiodothyronine in human serum or plasma.

The field of application is clinical laboratory diagnostics.

## 2. GENERAL INFORMATION

Triiodothyronine (T3) is a hormone with a molecular weight of 651 Da, 58% of which is iodine. Thyroid hormones thyroxin (T4) and 3,5,3′-triiodothyronine (T3) exert regulatory influences on growth, differentiation, cellular metabolism and development of skeletal and organ systems. T4 and T3 in blood are found both in free and bound form – mostly, they are bound to thyroxin binding globulin (TBG). Only free forms of T3 and T4 exert hormonal activity also their percentage is very low – 0.3% for T3 and 0.03% for T4.

The concentration of T3 is much less than that of T4 but its metabolic activity is about 3 times greater. About 80% of T3 is produced in peripheral tissues by deiodination of T4, and only 20% is secreted by thyroid gland. That is why in hypothyroid patients T3 level may for a long time remain on the lower limit of the normal range, because its loss may be compensated by enhanced conversion of T4 into T3.

Determination of T3 level is most useful in T3-hyperthyroidism because 5-10% of such patients do not show significant changes in T4 level while concentration of T3 is highly elevated. Elevated T3 levels are seen in early thyroid hypofunction, after intake of estrogens, oral contraceptives, heroin, methadone, during pregnancy.

Decreased concentrations of T3 are found in initial stage of hyperthyroidism, acute and subacute thyroiditis, after intake of androgens, dexamethasone, salycilates. Decreased concentrations of T3 are found in initial stage of hyperthyroidism, acute and subacute thyroiditis, after intake of androgens, dexamethasone, salycilates.

## 3. TEST PRINCIPLE

The determination of triiodothyronine is based on the competition principle of the enzyme immunoassay. On the inner surface of the microplate wells are immobilized specific rabbit polyclonal to T3 antibodies. T3 conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage T3 from the specimen competes with the conjugated T3 for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed.
- during the second stage, the complexes formed due to the reaction with the chromogen 3,3′,5,5′-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. The optical density in the microwell is inversely related to the quantity of the measured T3 in the serum specimen (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of T3 in the calibration samples.

## 4. KIT COMPONENTS

| Code of component | Symbol | Name | Volume | Qty, pcs. | Description |
|---|---|---|---|---|---|
| P211Z | SORB MTP | **Microplate** | - | 1 | 96-well polystyrene strip microplate coated with rabbit polyclonal antibodies to T3, ready to use; |
| C211Z | CAL 1 | **Calibrator C1** | 0.5 mL | 1 | Solution based on tris buffer (pH 7.2-7.4), free of T3, with preservative, ready to use (yellow liquid) |
| C211Z | CAL 2-5 | **Calibrators** | 0.5 mL | 4 | Solutions based on tris buffer (pH 7.2-7.4), containing 0,75; 1,5; 7,5 and 15 nmol/L of T3, with preservative, ready to use (blue liquids) |
| Q211Z | CONTROL | **Control serum** | 0.5 mL | 1 | Solution based on human plasma, containing of known T3 content, with preservative, ready to use (colourless liquid) |
| T211XZ | CONJ 2X | **Conjugate Concentrate** | 7 mL | 1 | Solution of T3 conjugated to the horseradish peroxidase; 2x concentrate (purple liquid) |
| ST211Z | DIL CONJ | **Conjugate Dilution Buffer** | 7 mL | 1 | Buffer solution with detergent ready to use (red liquid) |
| R055Z | SUBS TMB | **Substrate Solution** | 14 ml (мл) | 1 | Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid) |
| S008Z | BUF WASH 26X | **26x Concentrate Washing Solution** | 22 ml (мл) | 1 | Buffer solution with detergent, 26x concentrate (colourless liquid) |
| R050Z | STOP | **Stop Solution** | 14 ml (мл) | 1 | 5.0% solution of sulphuric acid; ready to use (colourless liquid) |
| The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.) | | | | | |

## 5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

- microplate photometer with 450 nm wavelength;
- shaker maintaining a speed of 500 rpm for +37 °C±2°C;
- automatic plate washer (optional);
- micropipettes with variable volume, range volume 5-1000 µL;
- graduated cylinder of 1000 mL capacity;
- distilled or deionized water;
- timer;
- vortex mixer;
- disposable gloves;
- absorbent paper.

## 6. WARNING AND PRECAUTIONS

*In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.*

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

**ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.**

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

## 7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below; do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

## 8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The T3 EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The T3 EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Concentrate, Conjugate Dilution Buffer, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;
- diluted Washing Solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

## 9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of Washing Solution concentrate and dilute it 26 times with distilled or deionized water.

9.4. Working conjugate solution preparation

Prepare a working conjugate solution by 2 dilutions of Conjugate Concentrate in Conjugate Dilution Buffer (eg, 450 µL of concentrate + 450 µL of Conjugate Dilution Buffer). In the case of partial use of the kit, take the necessary amount of Conjugate Concentrate and dilute it 2 times with Conjugate Dilution Buffer, since the working conjugate solution in a diluted form is not stored for a long time.

The spending of the components in case of partial use of the kit is given in the table:

| Quantity of strips | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Volume of the Washing Solution concentrate, mL | 1.8 | 3.6 | 5.4 | 7.2 | 9 | 10.8 | 12.6 | 14.4 | 16.2 | 18 | 19.8 | 22 |
| Volume of water, mL | 45 | 90 | 135 | 180 | 225 | 270 | 315 | 360 | 405 | 450 | 495 | 550 |
| Volume of Conjugate Concentrate, mL | 0.45 | 0.9 | 1.35 | 1.8 | 2.25 | 2.7 | 3.15 | 3.6 | 4.05 | 4.5 | 4.95 | 5.4 |
| Volume of Conjugate Dilution Buffer, mL | 0.45 | 0.9 | 1.35 | 1.8 | 2.25 | 2.7 | 3.15 | 3.6 | 4.05 | 4.5 | 4.95 | 5.4 |

## 10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each calibrator (CAL 1-5) and 2 wells for control serum (Q)).

10.2 Prepare Working conjugate solution as described in 9.4.

10.3 Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

*Note: during performing several independent series of tests, Calibrators, and Control Sample should be used each time.*

***Scheme of introduction of samples***

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | CAL1 | CAL1 | SAMP3 | SAMP3 | SAMP11 | SAMP11 | | | | | | |
| B | CAL2 | CAL2 | SAMP4 | SAMP4 | SAMP12 | SAMP12 | | | | | | |
| C | CAL3 | CAL3 | SAMP5 | SAMP5 | | | | | | | | |
| D | CAL4 | CAL4 | SAMP6 | SAMP6 | | | | | | | | |
| E | CAL5 | CAL5 | SAMP7 | SAMP7 | | | | | | | | |
| F | Q | Q | SAMP8 | SAMP8 | | | | | | | | |
| G | SAMP1 | SAMP1 | SAMP9 | SAMP9 | | | | | | | | |
| H | SAMP2 | SAMP2 | SAMP10 | SAMP10 | | | | | | | | |

10.4 Dispense **100 µL of Working conjugate solution** to all wells.

10.5 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C with continuous shaking 500 rpm**.

10.6 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.7 Add **100 µL of Substrate Solution** to all wells. The introduction of the substrate solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.8 Add **100 µL of Stop Solution** to all wells in the same order as the substrate solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.9 Read the optical density (OD) of the wells at 450nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.10 Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the T3 concentration in the calibrators nmol/L, (y) – OD versus T3 concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve. Adjust the concentration of CAL1 to an infinitesimally small value, for example, 0.001 nmol/L.

10.11 Determine the corresponding concentration of T3 in tested samples from the calibration curve.

## 11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

## 12. EXPECTED VALUES

Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for T3. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

*NOTE: values of T3 concentrations in the tested samples that are below the LoD (0.2 nmol/L) and also exceed the value of the upper calibrator (15 nmol/L) should be provided in the following form: «the T3 concentration of tested sample X is «lower than 0.2 nmol/L» or «higher than 15 nmol/L».*

The concentration values of the T3 EIA kit calibrators are expressed in nmol/L. To convert the concentration in ng/mL it is necessary to multiply by 0.65 the obtained concentration value in nmol/L.

1 nmol/L = 0.65 ng/mL

| Sex, age | Units, nmol/L | | Units alternative, ng/mL | |
|---|---|---|---|---|
| | Lower limit | Upper limit | Lower limit | Upper limit |
| Healthy donors | 1.2 | 3.2 | 0.8 | 2.1 |

## 13. PERFORMANCE CHARACTERISTICS

### 13.1. Analytical performance characteristics

13.1.1 Precision of Measurement

*Repeatability (Intra assay repeatability)* was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

| Sample | Concentration, nmol/L | CV, % |
|---|---|---|
| 1 | 2.32 | 9.16 |
| 2 | 1.45 | 9.66 |

*Reproducibility (Inter assay reproducibility)* was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

| Sample | Concentration, nmol/L | CV, % |
|---|---|---|
| 1 | 1.38 | 9.89 |
| 2 | 1.75 | 8.41 |

*Reproducibility between lots* was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

| Sample | Concentration1, nmol/L | Concentration2, nmol/L | Concentration3, nmol/L | CV, % |
|---|---|---|---|---|
| 1 | 2.12 | 2.02 | 2.27 | 13.9 |
| 2 | 1.56 | 1.44 | 1.81 | 15.6 |

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known T3 concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 0.75 –15 nmol/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest T3 concentration in the serum or plasma sample that is detected by the T3 EIA kit is no lower than 0.2 nmol/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for T3 EIA kit is 0.55 nmol/L.

3.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of T3 with other analytes is shown in the table:

| Analyte | Cross-reactivity, % |
|---|---|
| L-Thyroxin | 0.01 |
| D-Thyroxin | 0.04 |

## 14. REFERENCES

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3. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
4. Постанова КМУ від 02 жовтня 2013р. №754 «Про затвердження технічного регламенту щодо медичних виробів для діагностики in vitro».
5. НПАОП 85.14-1.09-81. Правила облаштування, техніки безпеки, виробничої санітарії, протиепідемічного режиму і особистої гігієни при роботі в лабораторіях (відділеннях, відділах) санітарноепідеміологічних установ системи Міністерства охорони здоров`я СРСР (НАОП 9.1.50-1.09-81)

## SAMPLES IDENTIFICATION PLAN

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LOT _____ DATE _____

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|---|---|
|  | Manufacturer |
|  | *In vitro* diagnistic medical device |
|  | Catalogue number |
|  | Use-by date |
|  | Batch code |
|  | Temperature limit |
|  | Contains sufficient for <n> tests |
|  | Caution |
|  | Consult instructions for use |
|  | Conformity Marking with technical regulations in Ukraine |
|  | Authorized representative in the European Community/European Union |
|  | CE Conformity Marking |

**For any issues related to operation of the kit and technical support, please contact by telefon number**

**+38 044 294-69-78**

**or write to:**

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**Instruction for use**
**A solid-phase enzyme immunoassay kit for the quantitative determination of thyroxin in human serum or plasma**

# T4 EIA

Catalogue number REF **K212**

Σ 96 For 96 determinations

IVD *In vitro* diagnostic medical device

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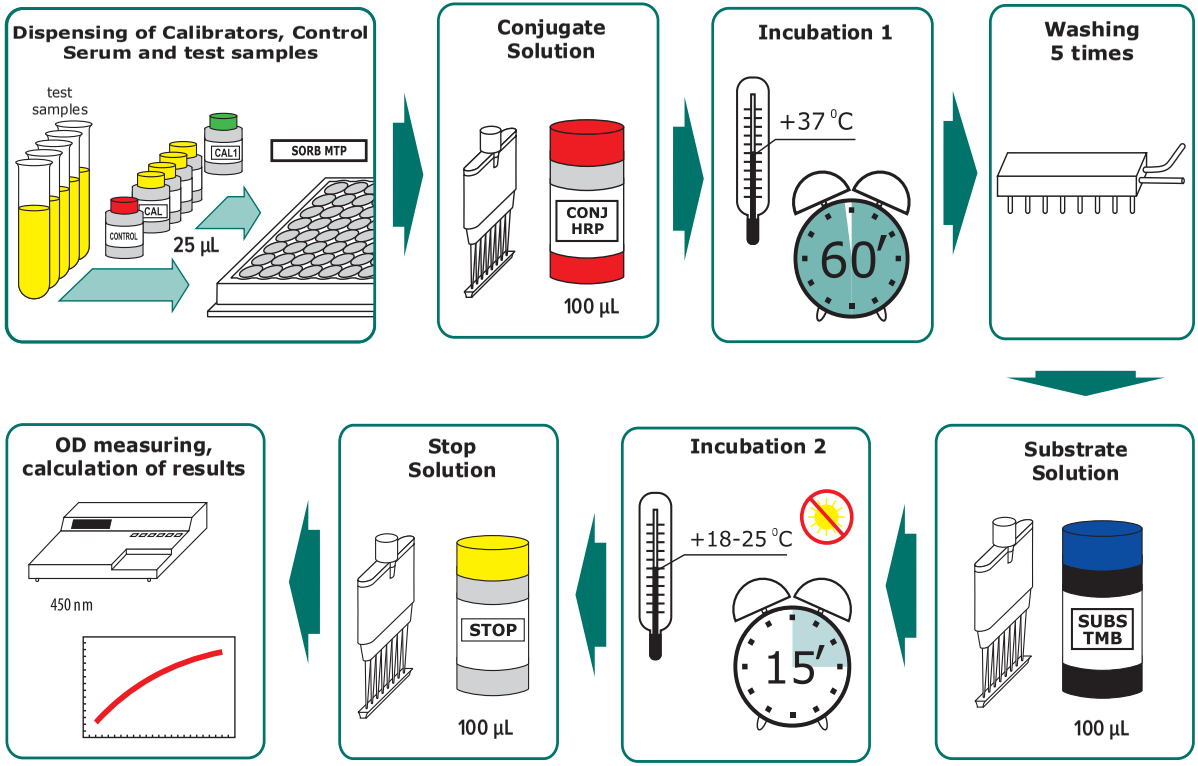
tel.:+ 49 911 931 639 67
E-mail: info@polmed.de
www.polmed.de

## ASSAY PROCEDURE




K212

**CONTENT**

# Instruction for use
# A solid-phase enzyme immunoassay kit for the quantitative determination of thyroxin in human serum or plasma
# T4 EIA

## 1. INTENDED USE

The T4 EIA kit is an enzyme immunoassay, intended for the quantitative determination of thyroxin in human serum or plasma.

The field of application is clinical laboratory diagnostics.

## 2. GENERAL INFORMATION

Thyroxine (T4) and triiodothyronine (T3) are hormones that are produced by the thyroid gland and circulate in the blood both free and bound - mainly with thyroxine-binding globulin (TBG). Only free T3 and T4 are characterized by Hormonal activity, but their share is very small: 0.03% of the total content for T4 and 0.3% - for T3. Concentration of T4 in serum blood is the most accepted indicator of thyroid gland function, which allows you to clearly distinguish between hyper-, hypo- and euthyroidism.

Increase of total T4 concentration is observed with hyperthyroidism, with pituitary tumors, with conditions with elevated TSH levels (pregnancy, acute or chronic active hepatitis, estrogen-secreting tumors or estrogen intake, genetically conditional increase), while taking oral contraceptives, heroin, methadone, thyroid drugs, TSH, thyroliberin.

Decrease of total T4 concentration is observed in hypothyroidism, panhypopituitarism, states of low levels of TSH (acromegaly, nephrotic syndrome, hypoproteinemia, chronic liver disease, androgen-secreting tumors, or androgens, genetically determined decrease), hemolysis, exercise, when taking amino salicylic and acetylsalicylic acids, glucocorticoids, sulfonamides, cholestyramine, reserpine, potassium iodide, triiodothyronine.

## 3. TEST PRINCIPLE

Determination of the thyroxine is based on competition principle of the enzyme immunoassay. Microwells plate is coated with specific murine monoclonal to thyroxine antibodies. Thyroxine conjugated to the horseradish peroxidase is used as enzyme conjugate. The analysis procedure includes two stages of incubation:

- during the first stage thyroxine from the specimen competes with the conjugated thyroxine for coating antibodies. As a result, a complex bounded to the solid phase and containing peroxidase is formed.
- during the second stage, the complexes formed due the reaction with the chromogen 3,3',5,5'-tetramethylbenzidine are visualized.

After stopping the reaction with a stop solution, the intensity of the color of the microwells is measured. Optical density in the microwell is inversely related to the quantity of the measured thyroxine in the specimen of the serum (plasma).

The concentration is determined according to the calibration graph of the dependence of the optical density on the content of thyroxine in the calibration samples.

## 4. KIT COMPONENTS

| Code of component | Symbol | Name | Volume | Qty, pcs. | Description |
|---|---|---|---|---|---|
| P212Z | SORB MTP | **Microplate** | - | 1 | 96-well polystyrene strip microplate coated with murine monoclonal antibodies to T4; ready to use |
| C212Z | CAL 1 | **Calibrator C1** | 0.5 mL | 1 | Solution based on human plasma, free of thyroxin, with preservative, ready to use (yellow liquid) |
| C212Z | CAL 2-5 | **Calibrators** | 0.5 mL | 4 | Solutions based on human plasma, containing 32; 64; 160 and 320 nmol/L of thyroxin, with preservative, ready to use (red liquids) |
| Q212Z | CONTROL | **Control Serum** | 0.5 mL | 1 | Solution based on human plasma, containing of known thyroxin content, with preservative, ready to use (colourless liquid) |
| T212XZ | CONJ | **Conjugate Solution** | 14 mL | 1 | Solution of thyroxin conjugated to the horseradish peroxidase; ready to use (red liquid) |
| R055Z | SUBS TMB | **Substrate Solution** | 14 mL | 1 | Tetramethylbenzidine (TMB) substrate solution; ready to use (colourless liquid) |
| S008Z | BUF WASH 26X | **26x Concentrate Washing Solution** | 22 mL | 1 | Buffer solution with detergent, 26x concentrate (colourless liquid) |
| R050Z | STOP | **Stop Solution** | 14 mL | 1 | 5.0% solution of sulphuric acid; ready to use (colourless liquid) |
| The kit also includes instruction for use, quality control data sheet and plate sealing tape (2 pcs.) | | | | | |

## 5. EQUIPMENT AND MATERIAL REQUIRED BUT NOT PROVIDED

– microplate photometer with 450 nm wavelength;
– dry thermostat for +37°C±2°C;
– automatic plate washer (optional);
– micropipettes with variable volume, range volume 5-1000 µL;
– graduated cylinder of 1000 mL capacity;
– distilled or deionized water;
– timer;
– vortex mixer;
– disposable gloves;
– absorbent paper.

## 6. WARNING AND PRECAUTIONS

*In order to prevent incorrect results, strictly follow the recommended order and duration of the analysis procedure.*

6.1. The kit is for *in vitro* diagnostic use only. For professional laboratory use.

6.2. Follow the rules mentioned below during the kit using:

- do not use kit beyond expire date;
- do not use the kit if its packaging is damaged;
- in order to avoid contamination, use new tips to pipette samples and reagents;
- use only verified equipment;
- close each vial with its own cap, after using the reagent;
- do not use components of other kits or reagents of other manufacturers;
- do not let wells dry after completing the rinsing step; immediately proceed to the next stage;
- avoid bubbles when adding reagents.

**ATTENTION! The TMB substrate solution is light sensitive. Avoid prolonged exposure of the component to light.**

6.3. Some kit components, such as stop solution, substrate solution, and washing solution, may cause toxic or irritant effects. If they get on the skin or mucosa, the affected area should be washed with plenty of running water.

6.4. All human products, including patient samples, should be considered potentially infectious. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guidelines or regulations.

6.5. The Calibrators and Control Serum included in the kit are negative for antibodies to HIV 1,2, hepatitis C virus and HBsAg, but the reagents should be considered as potentially infectious material and handled carefully.

6.6. Specimens must not contain any azide compounds, as they inhibit activity of peroxidase.

6.7. Wear protective gloves, protective clothing, eye protection, face protection.

6.8. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.

6.9. Safety Data Sheet for this product is available upon request directly from XEMA LLC.

6.10. Serious incidents related to the kit must be reported to the manufacturer, Authorized Representative, and to the Competent Authority of the EU member state(s) where the incident has occurred.

## 7. SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE OF SAMPLES

7.1. Blood sampling should be carried out from the cubital vein with a disposable needle using a vacuum blood sampling system. Serum or plasma specimens should be clearly labeled and identified. Serum must be separated from the clot as early as possible to avoid hemolysis of red blood cells. If there are any visible particles in the sample, they should be removed by centrifugation at 3000-5000 rpm for 20 minutes at room temperature or by filtration.

Don't use samples with high lipidemia, hemolysis as they may give false test results.

7.2. Specimen should be stored at +2…+8°C up to 3 days. Specimen held for a longer time, should be placed in a freezer at -15°C or below, do not refreeze/thaw samples.

7.3. For the transportation of samples, it is recommended to use triple packaging. The primary package is the labeled tube containing the sample. Secondary packaging is a polyethylene bag that is hermetically closed with a zip-lock. The outer packaging is a heat-insulating container, while the secondary packaging is placed in the outer packaging for transportation in the center of the thermal container. Frozen refrigerants are placed on the bottom, along the side walls of the thermal container, and cover the samples with them.

## 8. TRANSPORTATION AND STORAGE TERMS OF KIT, WASTE DISPOSAL

Information about the singularity storage conditions, transportation of the kit, and disposal of waste should be taken into account by all persons who participate in these processes.

8.1. Transportation

The T4 EIA kit should be transported in the manufacturer's packaging at +2...+8°C. Single transportation at the temperature up to 25ºC for 5 days is acceptable.

8.2. Storage

The T4 EIA kit should be stored in the manufacturer's packaging at +2...+8°C. Do not freeze.

The kit contains reagents sufficient for 96 determinations including Calibrators and Control Serum.

Once opened test-kit is stable for 2 months when stored properly as intended by manufacturer at 2-8°C.

In case of partial use of the kit, the components should be stored in the following way:

- strips that remain unused must be carefully sealed with the plate sealing tape and stored at +2...+8°C within 2 months;
- Substrate Solution, Stop Solution, and Washing Solution concentrate after opening the vial, can be stored tightly closed at +2...+8°C until the kit's shelf life;
- Conjugate Solution, Calibrators and Control Serum after opening the vial, can be stored tightly closed at +2...+8°C within 2 months;

  *NOTE: Single freezing of Calibrators and Control Serum in aliquots is allowed*
- diluted washing solution can be stored at room temperature (+18…+25°C) for up to 5 days or at +2…+8°C for up to 14 days.

Kits that were stored in violation of the storage condition cannot be used.

8.3. Disposal

Expired kit components, used reagents and materials, as well as residual samples must be inactivated and disposed of in accordance with legal requirements.

## 9. REAGENTS PREPARATION

9.1. All reagents (including microstrips) and test samples should be allowed to reach room temperature (+18…+25 °C) for at least 30 minutes before use.

9.2. Microplate preparation

Open the package with the microplate and install the required number of strips into the frame. Unused strips must be sealed with plate sealing tape to prevent moisture from affecting the plate's holes and placed back in the bag.

9.3. Washing Solution preparation

Add the contents of the 22 mL Washing Solution concentrate vial to 550 mL of distilled or deionized water and mix thoroughly. In case of partial use of the kit, take the necessary amount of washing solution concentrate and dilute it 26 times with distilled or deionized water.

The spending of the components in case of partial use of the kit is given in the table:

| Quantity of strips | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Volume of the Washing Solution concentrate, mL | 1.8 | 3.6 | 5.4 | 7.2 | 9 | 10.8 | 12.6 | 14.4 | 16.2 | 18 | 19.8 | 22 |
| Volume of water, mL | 45 | 90 | 135 | 180 | 225 | 270 | 315 | 360 | 405 | 450 | 495 | 550 |

## 10. ASSAY PROCEDURE

10.1 Put the desired number of strips into the frame based on the number of test samples in 2 replicates and 12 wells for Calibrators and Control Serum (2 wells for each calibrator (CAL 1-5) and 2 wells for control serum (Q)).

10.2 Dispense **25 µL of Calibrators and Control Serum as well as 25 µL of test serum/plasma samples** (SAMP) to the wells of the microplate according to the scheme below. The introduction of Calibrators, Control Serum and test samples should be carried out within 5 minutes to ensure equal incubation time for the first and last samples.

*Note: during performing several independent series of tests, Calibrators, and Control Sample should be used each time*.

***Scheme of introduction of samples***

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | CAL1 | CAL1 | SAMP3 | SAMP3 | SAMP11 | SAMP11 | | | | | | |
| B | CAL2 | CAL2 | SAMP4 | SAMP4 | SAMP12 | SAMP12 | | | | | | |
| C | CAL3 | CAL3 | SAMP5 | SAMP5 | | | | | | | | |
| D | CAL4 | CAL4 | SAMP6 | SAMP6 | | | | | | | | |
| E | CAL5 | CAL5 | SAMP7 | SAMP7 | | | | | | | | |
| F | Q | Q | SAMP8 | SAMP8 | | | | | | | | |
| G | SAMP1 | SAMP1 | SAMP9 | SAMP9 | | | | | | | | |
| H | SAMP2 | SAMP2 | SAMP10 | SAMP10 | | | | | | | | |

10.3 Add **100 µL of the Conjugate Solution** to all wells.

10.4 Carefully mix the contents of the microplate in a circular motion on a horizontal surface, cover strips with a plate sealing tape and incubate for **60 minutes at +37°C**.

10.5 At the end of the incubation period, remove and discard the plate cover. Aspirate and wash each well 5 times using an automatic washer or an 8-channel dispenser. For each washing, add 300 µL of Washing Solution (see 9.3) to all wells, then remove the liquid by aspiration or decantation. The residual volume of the Washing Solution after each aspiration or decantation should be no more than 5µL. After washing, carefully remove the remaining liquid from the wells on the absorbent paper. For the automatic washer/analyzer, the Washing Solution volume can be increased to 350 µL.

10.6 Add **100 µL of Substrate Solution** to all wells. The introduction of the substrate solution into the wells must be carried out within 2-3 minutes. Incubate the microplate in the dark **at room temperature (+18...+25°C) for 15 minutes**.

10.7 Add **100 µL of Stop Solution** to all wells in the same order as the substrate solution. After adding the Stop Solution, the contents of the wells turn yellow.

10.8 Read the optical density (OD) of the wells at 450nm using a microplate photometer within 5 minutes of adding the Stop Solution.

10.9 Plot a calibration curve in semi-logarithmic coordinates: (x) is the decimal logarithm of the T4 concentration in the calibrators nmol/L, (y) – OD versus T4 concentration (OD 450 nm). Manual or computerized data reduction is applicable at this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve. Adjust the concentration of CAL1 to an infinitesimally small value, for example, 0.001 nmol/L.

10.10 Determine the corresponding concentration of T4 in tested samples from the calibration curve.

## 11. TEST VALIDITY

The test run shall be considered valid if the OD of CAL1 is above 1.2, and the values of the Control Serum fall into the required range (see Quality control Data Sheet).

## 12. EXPECTED VALUES

12.1. Therapeutical consequences should not be based on results of IVD methods alone – all available clinical and laboratory findings should be used by a physician to elaborate therapeutically measures. Each laboratory should establish its own normal range for T4. Based on data obtained by XEMA, the following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying

*NOTE: values of T4 concentrations in the tested samples that are below the LoD (3.0 nmol/L) and also exceed the value of the upper calibrator (320 nmol/L) should be provided in the following form : «the T4 concentration of tested sample X is «lower than 3.0 nmol/L» or «higher than 320 nmol/L».*

12.2. The calibrators concentration values of the T4 EIA kit are expressed in nmol/L. To calculate concentrations in µg/dl, the received concentration value in nmol/L shall be multiplied by 0.0775.

**1 nmol/L = 0.0775 µg/dl**

| Sex, age | Units, nmol/L | | Units alternative, µg/dl | |
|---|---|---|---|---|
| | Lower limit | Upper limit | Lower limit | Upper limit |
| Healthy donors | 60 | 160 | 4.7 | 12.4 |
| Males | | | | |
| >61 yrs | 60 | 129 | 4.7 | 10.0 |
| Females | | | | |
| >61 yrs | 70 | 135 | 5.4 | 10.5 |
| Children | | | | |
| 1-5 yrs | 90 | 190 | 7.0 | 14.7 |
| 6-10 yrs | 83 | 170 | 6.4 | 13.2 |
| >10 yrs | 60 | 160 | 4.7 | 12.4 |

## 13. PERFORMANCE CHARACTERISTICS

### 13.1. Analytical performance characteristics

3.1.1 Precision of Measurement

*Repeatability (Intra assay repeatability)* was determined by evaluation the coefficient of variation (CV) for 2 different samples during 1 day in 24 replicates on one series of ELISA kit.

| Sample | Concentration, nmol/L | CV, % |
|---|---|---|
| 1 | 17.5 | 4.36 |
| 2 | 110.7 | 3.67 |

*Reproducibility (Inter assay reproducibility)* was determined by evaluating the coefficients of variation for 2 samples during 5 days in 8-replicate determinations.

| Sample | Concentration, nmol/L | CV, % |
|---|---|---|
| 1 | 16.4 | 1.17 |
| 2 | 111.1 | 5.43 |

*Reproducibility between lots* was investigated by testing samples for one day on three lots. Each sample was run in 8 replicates.

| Sample | Concentration1, nmol/L | Concentration2, nmol/L | Concentration3, nmol/L | CV, % |
|---|---|---|---|---|
| 1 | 14.59 | 13.67 | 15.39 | 5.92 |
| 2 | 116.23 | 114.53 | 120.13 | 2.45 |

13.1.2 Trueness

The trueness of measurement is the degree of closeness of the average value obtained from a large number of measurement results to the true value. The bias of the measurement result (bias of measurements) is the difference between the mathematical expectation of the measurement result and the true value of the measurand. The bias was calculated for each sample and it was determined that it corresponds to the specified limits of ± 10%.

13.1.3 Linearity

Linearity was determined using sera samples with known T4 concentration (low and high) and mixing them with each other and buffer solution in different proportions. According to the measurements, linear range of kit is 0.75–15 nmol/L ±10%.

13.1.4 Analytical sensitivity

Limit of detection (LoD) – the lowest T4 concentration in the serum or plasma sample that is detected by the T4 EIA kit is no lower than 3 nmol/L.

Limit of quantification (LoQ) – the lowest concentration of the analyte in the sample that is determined quantitatively with the declared trueness for T4 EIA kit is 32 nmol/L.

13.1.5 Analytical specificity

For the analysis result is not affected by the presence in the sample of bilirubin in a concentration of up to 0.21 mg/mL and hemoglobin in a concentration of up to 10 mg/mL.

The cross-reactivity of T4 with other analytes is shown in the table:

| Analyte | Cross-reactivity, % |
|---|---|
| T3 | 0.5 |
| D-Thyroxin | 30 |

## 14. REFERENCES

1. Helfand M et al. Screening for thyroid disease. Ann Intern Med 1990; 112:840.
2. Chopra, I.J. et al. A Radioimmunoassay of Thyroxine. J. Clinical EndocrinoL 1971; 33:865.
3. Young, D.S. et al. Effects of Drugs on Clinical Laboratory Tests. Clinical Chemistry 1975; 21: 3660.
4. Sterling, L. Diagnosis and Treatment of Thyroid Disease, Cleveland , CRC Press, P. 19 51 (1975).
5. Surks M.I. et al. American Thyroid Association guidelines for use of laboratory tests in thyroid disorders. JAMA 1990; 263:1529
6. Наказ МОЗ України №325 від 08.06.2015 «Про затвердження Державних санітарно-протиепідемічних правил і норм щодо поводження з медичними відходами».
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|---|---|
|  | Manufacturer |
|  | *In vitro* diagnistic medical device |
|  | Catalogue number |
|  | Use-by date |
|  | Batch code |
|  | Temperature limit |
|  | Contains sufficient for <n> tests |
|  | Caution |
|  | Consult instructions for use |
|  | Conformity Marking with technical regulations in Ukraine |
|  | Authorized representative in the European Community/European Union |
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**or write to:**

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Atlas Medical

Declaration Ref No: DC21-0249

Date: 15.10.2021

# CE Declaration of Conformity

| Name and address of Manufacturer | **Atlas Medical GmbH**<br>Ludwig-Erhard-Ring 3, 15827 Blankenefelde-Mahlow<br>Germany .<br>Tel: +49(0)33708355030<br>Email: info@atlas-medical.com |
|---|---|

Atlas Medical GmbH declared our his own responsibility that the following IVD medical devices:

| Product Code | Product Name | GMDN code |
|---|---|---|
| 8.17.003.0300 | Atlas Periodic Acid Schiff (PAS) Stain Kit, 3x100ml | 43587 |
| 8.17.004.0300 | Atlas Iron Stain Kit, 3x100ml | 43587 |
| 8.17.009.1000 | Atlas Gram Stain Kit | 43733 |
| 8.17.010.0750 | Atlas ZN (Kinyoun) stain pack , 3x250ml | 43587 |
| 8.15.144.0250 | Atlas ZN Decolouriser, 250 ml /Bottle | 43587 |
| 8.17.015.0500 | Atlas Diff-3 Stain. | 43587 |
| 8.17.016.1000 | Atlas Papanicolau Stain Pack. | 43587 |
| 8.17.110.0250 | Atlas Papanicolau Stain EA35, 250 ml /Bottle. | 43587 |
| 8.17.111.0250 | Atlas Papanicolau Stain EA36, 250 ml /Bottle | 43587 |
| 8.17.112.0250 | Atlas Papanicolau Stain EA65, 250 ml /Bottle. | 43587 |
| 8.17.114.0250 | Atlas Papanicolau Stain EA50, 250 ml /Bottle. | 43587 |
| 8.17.115.0250 | Atlas Papanicolau Stain OG6, 250 ml /Bottle. | 43587 |
| 8.17.014.1000 | Atlas Reticulocytes stain (Methylene Blue) , 1000 ml /Bottle | 43587 |
| 8.15.037.0250 | Atlas Eosin Y (1%) Stain, 250 ml/Bottle | 43587 |
| 8.15.038.0250 | Atlas Eosin Y (5%) Stain, 250 ml/Bottle. | 43587 |
| 8.15.041.0250 | Atlas Field Stain (Solution A), 250ml/Bottle | 43587 |
| 8.15.042.0250 | Atlas Field Stain (Solution B), 250ml/Bottle | 43587 |
| 8.15.043.0750 | Atlas Field Stain Kit 3x250ml (250ml Fixing Reagent , 250ml Eosin Reagent, 250ml Methylene Blue Reagent). | 43587 |
| 8.15.047.0250 | Atlas Giemsa Stain, 250 ml/Bottle. | 43587 |
| 8.15.059.0250 | Atlas Haematoxylin Harris Stain , 250 ml/Bottle | 43587 |
| 8.15.069.0250 | Atlas Leishman Stain , 250 ml/Bottle. | 43587 |
| 8.15.069.1000 | Atlas Leishman Stain , 1000 ml/Bottle. | 43587 |
| 8.15.074.0250 | Atlas Lugol's Iodine, 250 ml/Bottle. | 43587 |
| 8.15.078.0250 | Atlas May Grunwald Stain, 250 ml/Bottle. | 43587 |
| 8.15.105.0250 | Atlas New Methylene Blue for Reticulocytes, 250 ml/Bottle. | 43587 |
| 8.15.143.0250 | Atlas Wright's Stain, 250 ml/Bottle. | 43587 |
| 8.15.146.0100 | Atlas Immersion oil, 100 Bottle/Box | 43587 |

Atlas Medical

**Declaration Ref No: DC21-0249**

**Date: 15.10.2021**

Meets the essential requirments of In Vitro Diagnostic Medical Devices Directive 98/79/EC Annex I
And
EN ISO 13485 :2016 , EN 18113-1, -2,:2011, EN ISO 15223:2016
EN ISO 14971:2019, EN ISO 23640:2015, ISO 2859/1:1999,
EN ISO 13612:2002, EN ISO 13641:2002 , EN ISO 62366-1+A1:2020.

| | |
|---|---|
| **IVD Categorization** | Directive 98/79, Other IVDs (Non-annex II, non-self-test). |
| **Conformity Assesment Route** | Directive 98/79/EC , Annex III. |
| **Name , Address and Identification number of notified body** | N/A |

| | |
|---|---|
| **Date of issuance:** | 15. October.2021 |
| **Place** | Atlas Medical GmbH |
| **Signed by:**<br><br>**Position :** | Amani AL-Habahbeh<br><br>Regulatory Affairs Manager |

Atlas Medical GmbH
Ludwig - Erhard Ring 3
15827 Blankenfelde - Mahlow
Tel. (0049) 33708 - 355030

Declaration Ref No: DC21-0035

# CE Declaration of Conformity

**According to Annex III of the IVD Directive 98/79/EC**

We,

**Atlas Medical**
Head office: Ludwig-Erhard-Ring 3
Blankenfelde-Mahlow, Germany.
Tel: +49 - 33708 – 3550 30
Email: info@atlas-medical.com

Middle East Site: Sahab Free Zone Area, P. O. Box 212555, Amman, Jordan.
Tel.: +962 6 4026468
Fax: +962 6 4022588
Email: info@atlas-medical.com

Declare our responsibility that the following product:

**See Attached list**

- Comply with all essential requirements (Annexl) of the IVD Directive 98/79/EC. This compliance has been properly documented and covers the items listed in Annex I of the IVD Directive.
- This product is produced under Atlas quality system (ISO13485:2016) issued by GMED:
  **Certificate N$^{0}$**.: 36655 rev 1
  **Expiry Date**: October 8 $^{th}$.2023
- Comply with the essential requirements of following standards (EN 18113-1, -2,-4:2011, EN ISO 15223:2016 , EN ISO 23640:2015, EN ISO 14971:2019, ISO 2859/1:1999, EN ISO 13612:2002, EN ISO 13641:2002.

And
Intended for In-Vitro Professional use only.

**Manufacturer**
**Atlas Medical**
**Ludwig-Erhard-Ring 3**
**Blankenfelde-Mahlow , Germany.**

Atlas Medical
Quality Diagnostic Products

| Atlas Medical | Issue date | Date of review | Management approval | MRXDO10F.10 08.02.2011 |
|---|---|---|---|---|
| | March.2021 | 09.03.2021 | | |

Atlas Medical

# CE Declaration of Conformity

## According to Annex III of the IVD Directive 98/79/EC

| Product Description |
|---|
| 8.00.02.0.0100 : ASO Latex Kit, 100 Tests (4ml Latex, 2x1.0ml controls). |
| 8.00.00.0.0100: CRP Latex Kit, 100 Tests (4 ml Latex, 2x1.0 ml Controls) |
| 8.00.04.0.0100: RF Latex Kit, 100 Tests (4ml Latex, 2x1.0ml controls) |
| 8.00.17.0.0100: D-Dimer Latex Kit, 100 Tests |
| 8.00.13.0.0300 : Streptococcus Latex Kit, 6 Groups, 6x50 Tests (5x1.5ml Latex (A,B,C,G,F), 1x3ml Latex(D), 1x1.0ml Positive Control, 1x2ml Extraction Reagent E, 1x1.5ml Extraction Reagent 1, 1x1.5ml Extraction Reagent 2, 2x2.5ml Extraction Reagent 3, Stirring Sticks, Glass Slide). |
| 8.00.18.3.0500 : RPR Syphilis (Coarse Grain) Kit, 500 Tests (10 ml latex, 2x1ml control) Without card,stirring sticks. |
| 8.00.18.3.1000 RPR Carbon Antigen (Coarse Grain) Kit, 1000 Tests (Reagent only). |

Atlas Medical
Quality Diagnostic Products

Atlas Medical

Declaration Ref No: DC22-0015

Date : 13.05.2022

## CE Declaration of Conformity

We,

**Atlas Medical GmbH**
Head office: Ludwig-Erhard-Ring 3
15827 Blankenefelde-Mahlow Germany
Tel: +49(0)33708355030
Email: info@atlas-site.com

Middle East Site: : Sahab Industrial Zone Area, King Abdullah II Industrial City
Amman 11512, Jordan
Tel.: +962 6 4026468
Fax: +962 6 4022588
Email: info@atlas-medical.com

Declare our responsibility that the following product:

**Blood Grouping Reagents:**
**(Anti-A Monoclonal Reagent, Anti-B Monoclonal Reagent , Anti-AB Monoclonal Reagent and Anti-D IgG/IgG blend Reagent)**
**see the attached list of variants**
**That are classified as Annex II, list A**

Is produced under Atlas quality system (ISO13485: 2016) supported by GMED certificate and complies with the essential requirements of

**In Vitro Diagnostic Medical Devices Directive 98/79/EC**
**And**
**EN ISO 18113-1, -2 :2011, EN ISO 15223:2016**
**EN ISO 14971:2019, EN ISO 23640 :2015 , ISO 2859 :2017,**
**EN 13612:2002, EN 13641:2002 , EN 13975:2003,**
**EN ISO 13485:2016, EN 62366-1:2020**
And

Intended for In-Vitro Professional use only.

**Conformity Assessment Route:**
Annex IV.3 –Approval full Quality Assurance System.
Annex IV.4-EC Design Examination (of the product)

**Notified Body:**

| | | |
|---|---|---|
| G-MED | CE | 0459 |

**GMED, Laboratoire national de métrologie et d'essais**
**1 rue Gaston Boissier 75015 Paris**
**Tél. : 01 40 43 37 00 , TVA:FR 28 839 022 522**

**EC Certificates No.:**

- **CE Certificate of Approval full Quality Assurance System: 33540 rev4.**
- **CE Certificate Of EC Design Examination: 33544 rev3.**

| | | | | | |
|---|---|---|---|---|---|
| Atlas Medical GmbH | Start of CE Marking | Date of expiry | Name & Position | Signature | MRXDO10F.11 21.10.2013 |
| | 09th october 2017 | 26th May 2025 | Amani Al-habahbeh (RA Manager) | Amani | |

Atlas Medical

Atlas Medical

Declaration Ref No: DC22-0015

Date : 13.05.2022

| Product Code | Product Name | GMDN Code |
|---|---|---|
| 8.02.00.0.0010 | Anti-A Monoclonal Reagent (Titer: 1/512), 10ml/vial, 1 vial/Carton Box | 52532 |
| 8.02.00.1.0100 | Anti-A Monoclonal Reagent (Titer: 1/512), 10ml/vial. 10 vials / Plastic Pack | 52532 |
| 8.02.00.1.0180 | Anti-A Monoclonal Reagent (Titer: 1/512), 10ml/vial. 18 vials / Carton Box | 52532 |
| 8.02.01.0.0010 | Anti-B Monoclonal Reagent (Titer: 1/512), 10ml/vial, / Carton Box | 52538 |
| 8.02.01.1.0100 | Anti-B Monoclonal Reagent (Titer: 1/512), 10ml/vial, 10 vials / Plastic Pack | 52538 |
| 8.02.01.1.0180 | Anti-B Monoclonal Reagent (Titer: 1/512), 10ml/vial, 18 vials / Carton Box | 52538 |
| 8.02.02.0.0010 | Anti-AB Monoclonal Reagent (Titer: 1/512), 10ml/vial, 1 vial/ Carton Box | 46442 |
| 8.02.02.1.0100 | Anti-AB Monoclonal Reagent (Titer: 1/512), 10ml/vial, 10 vials/Plastic Pack | 46442 |
| 8.02.02.1.0180 | Anti-AB Monoclonal Reagent (Titer: 1/512), 10ml/vial, 18 vials/Carton Box | 46442 |
| 8.02.03.0.0010 | Anti-D IgG/IgM Blend Reagent (Titer: 1/128), 10ml/vial, 1 vial/ Carton Box | 52647 |
| 8.02.03.1.0100 | Anti-D IgG/IgM Blend Reagent (Titer: 1/128), 10ml/vial, 10 vials / Plastic Pack | 52647 |
| 8.02.03.1.0180 | Anti-D IgG/IgM Blend Reagent (Titer: 1/128), 10ml/vial, 18 vials / Carton Box | 52647 |
| 8.02.04.0.0010 | Anti-A Monoclonal Reagent (Titer: 1/256), 10ml/vial, 1 Vial/Carton Box | 52532 |
| 8.02.04.0.0100 | Anti-A Monoclonal Reagent (Titer: 1/256), 10ml/vial, 10 vials / Plastic Pack | 52532 |
| 8.02.05.0.0010 | Anti-B Monoclonal Reagent (Titer: 1/256), 10ml/vial, 1vial/Carton Box | 52538 |
| 8.02.05.0.0100 | Anti-B Monoclonal Reagent (Titer: 1/256), 10ml/vial, 10 vials /Plastic Pack | 52538 |
| 8.02.05.6.0030 | ABO Set (Anti-A (1/256), Anti-B (1/256), Anti-D (1/64)),3x10ml / plastic Pack | 45308 |
| 8.02.05.7.0020 | ABO Set: Anti-A (1/256), Anti-B (1/256), 2x10ml /Plastic Pack | 52695 |
| 8.02.06.0.0010 | Anti-AB Monoclonal Reagent (Titer: 1/256), 10ml/vial, 1vial/Carton Box | 46442 |
| 8.02.06.1.0100 | Anti-AB Monoclonal Reagent (Titer: 1/256), 10ml/vial,10 vials /Plastic Pack | 46442 |
| 8.02.06.1.0180 | Anti-AB Monoclonal Reagent (Titer: 1/256), 10ml/vial,18 vials / Carton Box | 45308 |
| 8.02.07.0.0010 | Anti-D IgG/IgM Blend Reagent (Titer: 1/64), 10ml/vial, 1Vial/ Carton Box | 52647 |
| 8.02.07.1.0100 | Anti-D IgG/IgM Blend Reagent (Titer: 1/64), 10ml/vial, 10 vials / Plastic Pack | 52647 |

| Atlas Medical GmbH | Start of CE Marking | Date of expiry | Name & Position | Signature. | MRXDO10F.11 21.10.2013 |
|---|---|---|---|---|---|
| | 09th october 2017 | 26th May 2025 | Amani Al-habahbeh (RA Manager) | | |

Atlas Medical
Quality Diagnostic Products

Atlas Medical

Declaration Ref No: DC22-0015

Date : 13.05.2022

| | | |
|---|---|---|
| 8.02.47.0.0030 | ABO Set (Anti-A (1/512), Anti-B (1/512), Anti-D (1/128)),3x10ml/Plastic Pack | 45308 |
| 8.02.47.1.0030 | ABO Set (Anti-A (1/256), Anti-B (1/256), Anti-D (1/64)), 3x10ml /Carton Box. | 45308 |
| 8.02.47.3.0030 | ABO Set (Anti-A (1/256), Anti-B (1/256), Anti-D (1/64)), 3x10ml /Plastic Pack | 45308 |
| 8.02.47.5.0030 | ABO Set (Anti-A (1/256), Anti-B (1/256), Anti-D (1/128)), 3x10ml/Plastic Pack | 45308 |
| 8.02.49.0.0040 | ABO Set (Anti-A (1/256), Anti-B (1/256), Anti-AB (1/256), Anti-D (1/64)), 4x10ml/Carton Box | 45308 |
| 8.02.49.2.0040 | ABO Set (Anti-A (1/256), Anti-B (1/256), Anti-AB (1/256), Anti-D (1/128)), 4 x 10ml, 4 vials/Plastic Pack | 45308 |
| 8.02.53.0.0040 | ABO Set (Anti-A (1/512), Anti-B (1/512), Anti-AB (1/512) Anti-D (1/128)), 4x10ml/Plastic Pack | 45308 |
| 8.02.53.1.0040 | ABO Set (Anti-A (1/512), Anti-B (1/512), Anti-AB (1/512) Anti-D (1/128)), 4x10ml, 4vials/Plastic Pack | 45308 |
| 8.02.70.0.0010 | Anti-A monoclonal reagent , Titer (1/1024), 10 ml/vial, 1Vial/ Carton Box | 52532 |
| 8.02.71.0.0010 | Anti-B Monoclonal reagent (Titer: 1/1024) , 10 ml/vial ,1Vial/ Carton Box | 52538 |
| 8.02.72.0.0010 | Anti-AB Monoclonal reagent (Titer: 1/1024) , 10 ml/vial , 1Vial/ Carton Box | 45308 |
| 8.02.85.0.0010 | Anti-D IgG/IgM Blend Reagent , Titer 1/256, 10ml/vial, 1Vial/ Carton Box | 52647 |

Atlas Medical
Quality Diagnostic Products

Atlas Medical

Date: 05/Jan/2023

# STATEMENT

We, Atlas Medical having a registered office at Ludwig-Erhard-Ring 3, 15827 Blankenfelde-Mahlow, Berlin, Germany assign SRL Sanmedico having a registered office at A. Corobceanu Street 7A, apt.9, Chisinau MD-2012, Moldova, as authorized representative in correspondence with the conditions of directive 98/79/EEC.

We declare that the company mentioned above is authorized to register, notify, renew or modify the registration of medical devices on the territory of the Republic of Moldova.

On Behalf of Manufacturer:

General Manager

Haya Amawi

Signature: _____

Date: 05.01.2023

Atlas Medical GmbH
Ludwig - Erhard Ring 3
15827 Blánkenfelde - Mahlow
Tel. (0049) 33708 - 355030

**Atlas Medical:** Ludwig-Erhard-Ring 3, 15827 Blankenfelde-Mahlow, Berlin, Germany,
Tel:+4933708355030

**Regulatory Office:** William James House, Cowley Rd, Cambridge, CB4 0WX, United Kingdom
Tel: +44 (0) 1223 858 910

**Middle East Site:** P.O Box 204, King Abdullah II Industrial Estate, Amman, 11512, Jordan
Tel: +962 6 4026468

Declaration Ref No: DC21-0207

Date: 06.09.2021

# CE Declaration of Conformity

| | |
|---|---|
| **Name and address of Manufacturer** | **Atlas Medical GmbH**<br>Ludwig-Erhard-Ring 3, 15827 Blankenfelde-Mahlow<br>Germany .<br>Tel: +49(0)33708355030<br>Email: info@atlas-medical.com |

Atlas Medical GmbH declared our his own responsibility that the following IVD medical devices:

| Product Code | Product Name | GMDN code |
|---|---|---|
| 8.04.21.0.0001 | Atlas H. pylori Antibody Rapid Test Device (Serum/Plasma), Individually Pouched, Bulk | 62029 |
| 8.04.21.0.0020 | Atlas H. pylori Antibody Rapid Test Device (Serum/Plasma), Individually Pouched, 20 Tests/Box | 62029 |
| 8.04.21.0.0030 | Atlas H. pylori Antibody Rapid Test Device (Serum/Plasma), Individually Pouched, 30 Tests/Box | 62029 |
| 8.04.20.0.0001 | Atlas H. pylori Antibody Rapid Test Device (Whole blood/Serum/Plasma), Individually Pouched, Bulk | 62029 |
| 8.04.20.0.0020 | Atlas H. pylori Antibody Rapid Test Device (Whole blood/Serum/Plasma), Individually Pouched, 20 Tests/Box | 62029 |
| 8.04.20.0.0030 | Atlas H. pylori Antibody Rapid Test Device (Whole blood/Serum/Plasma), Individually Pouched, 30 Tests/Box | 62029 |

Meets the essential requirments of In Vitro Diagnostic Medical Devices Directive 98/79/EC Annex I

And

EN ISO 13485 :2016 , EN 18113-1, -2,:2011, EN ISO 15223:2016
EN ISO 14971:2019, EN ISO 23640:2015, ISO 2859/1:1999,
EN ISO 13612:2002, EN ISO 13641:2002 , EN ISO 62366-1+A1:2020.

| | |
|---|---|
| **IVD Categorization** | Directive 98/79, Other IVDs (Non-annex II, non-self-test). |
| **Conformity Assesment Route** | Directive 98/79/EC , Annex III. |
| **Name , Address and Identification number of notified body** | N/A |

| | |
|---|---|
| **Date of issuance:** | 06.September.2021 |
| **Place** | Atlas Medical GmbH |
| **Signed by:**<br><br>**Position :** | Amani AL-Habahbeh<br><br>Regulatory Affairs Manager |

Atlas Medical GmbH
Ludwig - Erhard Ring 3
15827 Blankenfelde - Mahlow
Tel. (0049) 33708 - 355030



MRXDO10F.11
11.08.2021

# CERTIFICAT
*CERTIFICATE OF REGISTRATION*
**N° 36655 rev.2**

**GMED certifie que le système de management de la qualité développé par**

*GMED certifies that the quality management system developed by*

## ATLAS MEDICAL GmbH
**Ludwig-Erhard-Ring 3**
**15827 Blankenfelde-Mahlow GERMANY**

**pour les activités**
*for the activities*

**Conception et développement, fabrication et vente de dispositifs médicaux de diagnostic in vitro .**

*Design and Development, Manufacturing and Sales of in vitro diagnostic medical devices.*

**réalisées sur le(s) site(s) de**
*performed on the location(s) of*

**Voir addendum**

*See addendum*

**est conforme aux exigences des normes internationales**
*complies with the requirements of the international standards*

## ISO 13485: 2016

**Début de validité** */ Effective date* **October 9th, 2023 (included)**
**Valable jusqu'au** */ Expiry date :* **October 8th, 2026 (included)**
**Etabli le** */ Issued on :* **October 9th, 2023**

DocuSigned by:
Beatrice Lys
EF33BDA9BAA04A3...

**On behalf of the President**
**Béatrice LYS**
**Technical Director**

cofrac
CERTIFICATION DE SYSTEMES DE MANAGEMENT
Accréditation n°4-0608
Liste des sites accrédités et portée disponible sur www.cofrac.fr

GMED N° 36655–2
Ce certificat est délivré selon les règles de certification GMED / This certificate is issued according to the rules of GMED certification

Renouvelle le certificat 36655-1



**GMED** • Société par Actions Simplifiée au capital de 300 000 € • Organisme Notifié/Notified Body n° 0459
Siège social : 1, rue Gaston Boissier - 75015 Paris • Tél. : 01 40 43 37 00 • gmed.fr

**Ce certificat couvre les activités et les sites suivants :**
*This certificate covers the following activities and sites:*

**French version :**
**Conception et développement, fabrication et vente de dispositifs médicaux de diagnostic *in vitro* à usage professionnel et/ ou d'autodiagnostic, dans les domaines du groupage sanguin, de la microbiologie, de la biochimie, de la toxicologie, de l'oncologie, de la cardiologie, de l'histologie, de l'endocrinologie et des maladies infectieuses, dans les techniques d'Agglutination/ ELISA/ Tests rapides/ Colorimétrie/ Disques antibiotiques.**

*English version:*
*Design and Development, Manufacturing and Sales of in vitro diagnostic medical devices for professional use and/or for self-testing, in the field of Immunohematology, Microbiology, Biochemistry, Toxicology, Oncology, Cardiology, Histology, Endocrinology Biosensors and Infectious diseases, in techniques of Agglutination/ ELISA/ Rapid tests/ Colorimetry/Antibiotic disks.*

**ATLAS MEDICAL GmbH**
**Ludwig-Erhard-Ring 3**
**15827 Blankenfelde-Mahlow**
**GERMANY**

French version:
**Siège social, responsable de la mise sur le marché**
*English version*:
*Headquarter, legal manufacturer*

**********

**Sahab Industrial Zone Area**
**King Abdullah II Industrial City**
**Amman 11512**
**JORDAN**

French version:
**Conception, fabrication et contrôle final**
*English version*:
*Design, manufacture and final control*

**********

**2 sites** / *2 sites*

DocuSigned by:
Beatrice Lys
FF33BDA9BAA04A3...

**On behalf of the President**
**Béatrice LYS**
**Technical Director**

Atlas Medical

# Certificate of Analysis for Blood Grouping Kit

## 1- Product Identification:

| | | | |
|---|---|---|---|
| **Product Name : Anti-D IgM Monoclonal Reagent** | **Catalog No. (Variant Code ) : 8.02.03.7.0001** | **Item Dispense #: 4211** | **Minimal Titer Accepted: 1/128** |
| **Lot #: 23102105** | **Mfg. Date: NA** | **Exp. Date: 2025/10/23** | |

## 2- Sampling Plan:

| Date | QC Test Method Used | Inspection level | AQL | Determine the following by referring to Sampling Plan Sheet | | | |
|---|---|---|---|---|---|---|---|
| | | | | Sample Size Code Letter | Sample Size (Test QTY) | Accepted | Rejected |
| 28.10.2023 | **F13D** | Physical Inspection: S-I | 1.0 | **B** | **3** | **0** | **1** |
| 28.10.2023 | **F13D** | Biochemical Inspection: One sample | **Not Applicable** | | | | |

## 3- Physical Check:

| Applicable Test Type | Inspected Item and/or Criteria | Inspection Results |
|---|---|---|
| ➢ Kit Assembly: | All components of the kit are present according to the outer label | ■ Pass ☐ Fail |
| ➢ Item Color & Status: | Anti-A: Blue – Liquid NA | ☐ Pass ☐ Fail |
| | Anti-B: Yellow – Liquid NA | ☐ Pass ☐ Fail |
| | Anti-D: Yellowish – Liquid | ■Pass ☐ Fail |
| | Anti-AB: Yellowish – Liquid NA | ☐ Pass ☐ Fail |
| ➢ Item Size/ Reagent Size is compatible with that requested in Item Dispense: | Anti-A NA | ☐ Pass ☐ Fail |
| | Anti-B NA | ☐ Pass ☐ Fail |
| | Anti-D 10 ml | ■Pass ☐ Fail |
| | Anti-AB NA | ☐ Pass ☐ Fail |
| ➢ Labels: | Correct label orientation | ■ Pass ☐ Fail |
| | Correct label position | ■Pass ☐ Fail |
| | Clear printing | ■Pass ☐ Fail |
| ➢ Package Insert: | Clear printing and correct folding | ■ Pass ☐ Fail |
| | Correct code, version and brand as mentioned in Item Dispense | ■Pass ☐ Fail |
| | Address as mentioned on box design | ■Pass ☐ Fail |
| ➢ Closing Cap: | No leakage and closed well | ■Pass ☐ Fail |
| ➢ Dropper Coloring / Titer **(CE Blood Grouping):** | Anti A (High titer (1/512): Blue cap with black bulb | ☐ Pass ☐ Fail |
| | Anti A (Low titer (1/256): Blue cap with grey bulb | ☐ Pass ☐ Fail |
| | Anti B (High titer (1/512): Yellow cap with black bulb | ☐ Pass ☐ Fail |
| | Anti B (Low titer (1/256): Yellow cap with grey bulb | ☐ Pass ☐ Fail |

| | | |
|---|---|---|
| | Anti AB (High titer (1/512): Grey cap with black bulb | ☐ Pass ☐ Fail |
| | Anti AB (Low titer (1/256): Grey cap with grey bulb | ☐ Pass ☐ Fail |
| | Anti D (High titer (1/128): Black cap with black bulb | ☐ Pass ☐ Fail |
| | Anti D (Low titer (1/64): Black cap with grey bulb | ☐ Pass ☐ Fail |
| ➢ Dropper Coloring / Titer **(None CE Blood Grouping):** | Anti A (High titer (1/512): White cap with black bulb | ☐ Pass ☐ Fail |
| | Anti A (Low titer (1/256): White cap with white bulb | ☐ Pass ☐ Fail |
| | Anti B (High titer (1/512): White cap with black bulb | ☐ Pass ☐ Fail |
| | Anti B (Low titer (1/256): White cap with white bulb | ☐ Pass ☐ Fail |
| | Anti AB (High titer (1/512): White cap with black bulb | ☐ Pass ☐ Fail |
| | Anti AB (Low titer (1/256): White cap with white bulb | ☐ Pass ☐ Fail |
| | Anti D (High titer (1/128): Black cap with white bulb | ☐ Pass ☐ Fail |
| | Anti D (Low titer (1/64): Gray cap with white bulb | ☐Pass ☐ Fail |
| | Anti D (IgM) (Low titer (1/64)): Grey cap with Black bulb | ☐Pass ☐ Fail |
| | Anti D (IgM) (High titer (1/128)): Black cap with black bulb | ■ Pass ☐ Fail |
| | Anti D (IgG) (Low titer (1/64)): Grey cap with black bulb | ☐Pass ☐ Fail |
| | Anti D (IgG) (High titer (1/128)): Black cap with black bulb | ☐ Pass ☐ Fail |
| ➢ Dropper Coloring / Titer **(Real Titer (256) / Non CE Blood Grouping):** | Anti A (White cap with white bulb) | ☐ Pass ☐ Fail |
| | Anti B (White cap with white bulb) | ☐ Pass ☐ Fail |
| | Anti AB (White cap with white bulb) | ☐ Pass ☐ Fail |
| ➢ Dropper Function: | Able to withdraw the reagent | ■ Pass ☐ Fail |
| ➢ Quantity/Kit: | Compatible with the quantity mentioned in the outer label<br>• Record the QTY/Kit: 2/1……. | ■Pass ☐ Fail |
| ➢ Final Result: | ■Pass ☐ Fail; justify …….. | |
| Done by QC Officer/Supervisor (Sign.): rayan | Date: 28/10/2023 | Time: 10:30 |

### 4- Biochemical Check:

**A. Direct Slide Method**: Interpret the results by referring to Table (01)

| Pipette #:**157** | | | | Pipette Code: E21PiQ157 | | | |
|---|---|---|---|---|---|---|---|
| **Anti A** | | **Anti –B** | | **Anti-AB** | | **Anti-D** | |
| A (lot No: ) | | B ( Lot no: ) | | AB (Lot no: ) | | O+(Lot no: Fresh sample ) | |
| Reaction time | Agglutination strength | Reaction time | Agglutination strength | Reaction time | Agglutination strength | Reaction time | Agglutination strength |
| NA | NA | NA | NA | NA | NA | 2 Sec | +3 |
| ➢ Final Result: | | ■ Pass ☐ Fail; justify …….. | | | | | |
| Done by QC Officer/Supervisor (Sign.): rayan | | Date: 28/10/2023 | | Time: 09:22 | | | |

** Testing by Direct tube method: (+4)

**B. Sensitivity test**

| Pipette #: **157** | | | | Pipette Code: E21PiQ157 | | |
|---|---|---|---|---|---|---|
| **Type of Test** | | | **Anti-A** | **Anti-B** | **Anti-AB** | **Anti-D** |
| Sensitivity | Tube Test | Type of Cell | A (Lot no: NA ) | B ( Lot no: NA ) | A (Lot no: )<br>B (Lot no ) | O+ (Lot no) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Method | Suspension | | | | | | | | |
| | | Result | 1:2 | NA | 1:2 | NA | 1:2 | NA | 1:2 | NA |
| | | | 1:4 | NA | 1:4 | NA | 1:4 | NA | 1:4 | NA |
| | | | 1:8 | NA | 1:8 | NA | 1:8 | NA | 1:8 | NA |
| | | | 1:16 | NA | 1:16 | NA | 1:16 | NA | 1:16 | NA |
| | | | 1:32 | NA | 1:32 | NA | 1:32 | NA | 1:32 | NA |
| | | | 1:64 | NA | 1:64 | NA | 1:64 | NA | 1:64 | NA |
| | | | 1:128 | NA | 1:128 | NA | 1:128 | NA | 1:128 | NA |
| | | | 1:256 | NA | 1:256 | NA | 1:256 | NA | 1:256 | NA |
| | | | 1:512 | NA | 1:512 | NA | 1:512 | NA | 1:512 | NA |
| | | | 1:1024 | NA | 1:1024 | NA | 1:1024 | NA | | |
| ➢ Final Result: | | ☐ Pass ☐ Fail; justify …….. | | | | | | | | |
| Done by QC Officer/Supervisor (Sign.): ……NA…….. Date: ……NA……. Time: ……NA…….. | | | | | | | | | | |

**Table (01)**

| Blood Grouping Reagents | Control Cell | Reaction Time | Agglutination Strength |
|---|---|---|---|
| Anti-A | A - Cell | Up to 3 second | +4 |
| Anti-B | B-Cell | Up to 3 second | +4 |
| Anti-AB | A B-Cell | Up to 3 second | +3/+4 |
| Anti –D | O RH positive cell | Up to 5 second | +3 |

| **Final Conclusion: ■ Pass ☐ Fail** | |
|---|---|
| **Final QC Manager Approval (Signature):** Tasneem | **Date:28/10/2023** |

**QC Release Stamp:**

Atlas Medical

# ASO LATEX KIT

IVD For in -*vitro* diagnostic and professional use only

2°C 8°C Store at 2-8°C. CE

**INTENDED USE**

ATLAS ASO latex Test is used for the qualitative and semi-quantitative measurement of antibodies to Antistreptolysin-O in human serum.

**INTRODUCTION**

The group A ß-hemolytic streptococci produce various toxins that can act as antigens. One of these exotoxins streptolysin-O, was discovered by Todd in 1932.

A person infected with group A hemolytic streptococci produces specific antibodies against these exotoxins, one of which is antistreptolysin-O. The quantity of this antibody in a patient's serum will establish the degree of infection due to the hemolytic streptococcal.

The usual procedure for the determination of the antistreptolysin titer is based on the inhibitory effect that the patient's serum produces on the hemolytic power of a pre-titrated and reduced streptolysin-O. However, the antigen-antibody reaction occurs independently of the hemolytic activity of streptolysin-O. This property enables the establishment of a qualitative and quantitative test for the determination of the antistreptolysin-O by agglutination of latex particles on slide.

**PRINCIPLE**

ASO test method is based on an immunologic reaction between streptococcal exotoxins bound to biologically inert latex particles and streptococcal antibodies in the test sample. Visible agglutination occurs when increased antibody level is present in the test specimen.

**MATERIALS**

**MATERIALS PROVIDED**

- ASO Latex Reagent: Latex particles coated with streptolysin O, pH, 8,2. Preservative.
- ASO Positive Control (Red cap): Human serum with an ASO concentration > 200 IU/mL.Preservative.
- ASO Negative Control (Blue cap) Animal serum. Preservative
- Glass Slide.
- Stirring Sticks.

**Note: This package insert is also used for individually packed reagent.**

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Mechanical rotator with adjustable speed at 80-100 r.p.m.
- Vortex mixer.
- Pippetes 50 µL.
- Glycine Buffer-20x (1000 mmol/l): add one part to nineteen parts of distilled water before use.

**Packaging contents**

REF **8.00.02.0.0100 (1x4ml Latex Reagent, 1x1ml positive control, 1x1ml negative control)**

**PRECAUTIONS**

- All reagents contain 0.1 %(w/v) sodium azide as a preservative.
- Protective clothing should be worn when handling the reagents.
- Wash hands and the test table top with water and soap once the testing is done.
- Reagents containing sodium azide may be combined with copper and lead plumbing to form highly explosive metal azides. Dispose of reagents by flushing with large amounts of water to prevent azide buildup.
- For In Vitro diagnostic use.
- Components prepared using human serum found negative for hepatitis B surface antigen (HBsAg), HCV and antibody to HIV (1/2) by FDA required test. However, handle controls as if potentially infectious.
- Accuracy of the test depends on the drop size of the latex reagent (40µl). Use only the dropper supplied with latex and hold it perpendicularly when dispensing.
- Use a clean pipette tip and stirring stick for each specimen, and glass slides should be thoroughly rinsed with water and wiped with lint-free tissue after each use.
- Check reactivity of the reagent using the controls provided.
- Do not use these reagents if the label is not available or damaged.
- Do not use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.

**REAGENT PREPARATION:**

The ASO Latex reagent is ready to use. No preparation is required. Mix gently before use to ensure a uniform suspension of particles.

**STORAGE AND STABILITY**

- Reagents are stable until specified expiry date on bottle label when stored refrigerated (2-8°C).
- **DO NOT FREEZE.**
- The ASO Latex Reagent, once shaken must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal.
- Do not use the latex reagent or controls if they become contaminated.
- Always keep vials in vertical position. If the position is changed, gently mix to dissolve aggregates that may be present.
- Reagents deterioration: Presence of particles and turbidity.

**SAMPLES**

- Use fresh serum collected by centrifuging clotted blood.
- If the test cannot be carried out on the same day, store the specimen for 7 days at 2-8°C and for 3 months at -20°C.
- Samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolyzed or lipemic samples.
- **DO NOT USE PLASMA.**

**PROCEDURE**

**Qualitative method**

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place (40 µL) of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Mix the ASO-latex reagent vigorously or on a vortex mixer before using and add one drop (40 µL) next to the sample to be tested.
4. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5. Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test is read later than two minutes.

**Semi-quantitative method**

1. Make serial two-fold dilutions of the sample in 9 g/L saline solution.
2. Proceed for each dilution as in the qualitative method.

**QUALITY CONTROL**

- Positive and Negative Controls should be included in each test batch.
- Acceptable performance is indicated when a uniform milky suspension with no agglutination is observed with the ASO Negative Control and agglutination with large aggregates is observed with the ASO Positive Control.

**CALCULATIONS**

The approximate ASO concentration in the patient sample is calculated as follows:

200 x ASO Titer = IU/mL

**READING AND INTERPRETATION**

Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator. The presence of agglutination indicates an ASO concentration equal or greater than 200 IU/mL

The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result.

**REFERENCE VALUES**

Up to 200 IU/mL(adults) and 100 IU/mL (children < 5 years old). Each laboratory should establish its own reference range.

**PERFORMANCE CHARACTERISTICS**

***Analytical sensitivity:***

200 (±50) IU/ml.

***PROZONE EFFECT***

No prozone effect was detected up to 1500 IU/ml.

***SENSITIVITY***

98%.

***SPECIFICITY***

97%.

**INTERFERENCES**

**NON-INTERFERING SUBSTANCES:**

- Hemoglobin (10 g/L)
- Bilirubin(20 mg/dL)
- Lipids (10 g/L)
- Rheumatoid factors (300 IU/mL)
- Other substances may interfere.

**LIMITATIONS**

- Reaction time is critical. If reaction time exceeds 2 minutes, drying of the reaction mixture may cause false positive result.
- Freezing the ASO Latex Reagent will result in spontaneous agglutination.
- Intensity of agglutination is not necessarily indicative of relative ASO concentration; therefore, screening reactions should not be graded.
- False positive results may be obtained in conditions such as, rheumatoid arthritis, scarlet fever, tonsilitis, several streptococcal infections and healthy carriers.
- Early infections and children from 6 months to 2 years may cause false negative results. A single ASO determination does not produce much information about the actual state of the disease.
- Titrations at biweekly intervals during 4 or 6 weeks are advisable to follow the disease evolution.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

**REFERENCES**


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**Website:** www.atlas-medical.com



**PPI2325A01**
**Rev A (05.01.2023)**


| | | | |
|---|---|---|---|
| REF | Catalogue Number | | Temperature limit |
| IVD | *In Vitro* diagnostic medical device | | Caution |
| | Contains sufficient for <n> tests and Relative size | | Consult instructions for use (IFU) |
| LOT | Batch code | | Manufacturer |
| | Fragile, handle with care | | Use-by date |
| | Manufacturer fax number | | Do not use if package is damaged |
| | Manufacturer telephone number | | Date of Manufacture |
| | Keep away from sunlight | | Keep dry |
| CONTROL+ | Positive control | CONTROL- | Negative control |

Atlas Medical

# CRP LATEX KIT

IVD For *in -vitro* diagnostic and professional use only

2°C 8°C Store at 2-8°C. CE

## INTENDED USE

CRP Latex kit is used to measure the CRP in human serum qualitatively and semi- quantitatively.

## INTRODUCTION

C-reactive protein (CRP), the classic acute-phase of human serum, is synthesized by hepatocytes. Normally, it is present only in trace amounts in serum, but it can increase as much as 1,000-fold in response to injury or infection. The clinical measurement of CRP in serum therefore appears to be a valuable screening test for organic disease and a sensitive index of disease activity in inflammatory, infective and ischemic conditions. MacLeod and Avery found that antibody produced against purified CRP provided a more sensitive test than the C-polysaccharide assay. Since that time a number of immunological assays have been devised to measure CRP such as capillary precipitation, double immunodiffusion and radical immunodiffusion.

The CRP reagent kit is based on the principle of the latex agglutination assay described by Singer and Plotz. The major advantage of this method is the rapid two (2) minute reaction time.

## PRINCIPLE

The CRP reagent kit is based on an immunological reaction between CRP Antisera bound to biologically inert latex particles and CRP in the test specimen. When serum CRP equal or greater than the Reagent sensitivity (Indicated on the label of the latex vial) the visible agglutination occurs.

## MATERIALS

### MATERIALS PROVIDED

- CRP Latex Reagent: Latex particles coated with goat IgG anti-human CRP (approximately 1 %), pH 8.2 MIX WELL BEFORE USE.
- CRP Positive Control Serum (Red Cap): A stabilized pre-diluted human serum containing >20mg/L CRP.
- CRP Negative Control Serum (Blue Cap): A stabilized pre-diluted animal serum.
- Glass Slides.
- Stirring Sticks.
- Package insert.

**NOTE: This package insert is also used for individually packed reagent.**

### MATERIALS REQUIRED BUT NOT PROVIDED

- Mechanical rotator with adjustable speed at 80-100 r.p.m.
- Vortex mixer.
- Pippetes 50 µL.
- Glycine Buffer 20X (1000 mmol/L): add one part to nineteen parts of distilled water before use.

### PACKAGING CONTENTS

REF **8.00.00.0.0100 (1x4ml Latex Reagent, 1x1ml positive control, 1x1ml negative control)**

### PRECAUTIONS

- All reagents contain 0.1 %(w/v) sodium azide as a preservative.
- Protective clothing should be worn when handling the reagents.
- Wash hands and the test table top with water and soap once the testing is done.
- Reagents containing sodium azide may be combined with copper and lead plumbing to form highly explosive metal azides. Dispose of reagents by flushing with large amounts of water to prevent azide buildup.
- For In *Vitro* diagnostic use.
- Components prepared using human serum found negative for hepatitis B surface antigen (HBsAg), HCV and antibody to HIV (1/2) by FDA required test. However, handle controls as if potentially infectious.
- Accuracy of the test depends on the drop size of the latex reagent (40µl). Use only the dropper supplied with latex and hold it perpendicularly when dispensing.
- Use a clean pipette tip and stirring stick for each specimen, and glass slides should be thoroughly rinsed with water and wiped with lint-free tissue after each use.
- Check reactivity of the reagent using the controls provided.
- Do not use these reagents if the label is not available or damaged.
- Do not use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.

### REAGENT PREPARATION:

The CRP Latex reagent is ready to use. No preparation is required. Mix gently before use to ensure a uniform suspension of particles.

### STORAGE AND STABILITY

- Reagents are stable until specified expiry date on bottle label when stored refrigerated (2 - 8°C).
- **DO NOT FREEZE.**
- The CRP latex reagent, once shaken must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal.
- Do not use the latex reagent or controls if they become contaminated.
- Always keep vials in vertical position. If the position is changed, gently mix to dissolve aggregates that may be present.
- Reagents deterioration: Presence of particles and turbidity.

### SPECIMEN COLLECTION AND STORAGE

- Use fresh serum collected by centrifuging clotted blood.
- If the test cannot be carried out on the same day, store the specimen for 7 days at 2-8°C and for 3 months at -20°C.
- Samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolyzed or lipemic samples.
- Do not use plasma.

### PROCEDURE

**A. QUALITATIVE TEST:**

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place (40 µL) of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Mix the CRP-latex reagent vigorously or on a vortex mixer before using and add one drop (40 µL) next to the samples to be tested.
4. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5. Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test is read later than two minutes.

**B. SEMI-QUANTITATIVE TEST:**

1. Make serial two-fold dilutions of the sample in 9 g/L saline solution.
2. Proceed for each dilution as in the qualitative method.

### QUALITY CONTROL

- Positive and Negative controls are recommended to monitor the performance of the procedure, as well as a comparative pattern for a better result interpretation.
- All result different from the negative control result, will be considered as a positive.

### READING AND INTERPRETATION

Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator.

The presence of agglutination indicates a CRP concentration equal or greater than the reagent sensitivity (mg/L CRP) (indicated on the label of the latex vial).

The titer, in semi-quantitative method, is defined as the highest dilution showing a positive result.

### CALCULATIONS

The approximate CRP concentration in the patient sample is calculated as follows:

Sensitivity (Indicated on the label of the latex vial)
x CRP Titer = mg/L

### INTERFERENCES

**NONE INTERFERING SUBSTANCES:**

- Hemoglobin (10 g/dl)
- Bilirubin (20 mg/dl)
- Lipids (10 g/L)
- Other substances interfere, such as RF (100IU/ml).

### NOTE

- High CRP concentration samples may give negative results. Retest the sample again using a drop of 20µl.
- The strength of agglutination is not indicative of the CRP concentration in the samples tested.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

### LIMITATIONS

1. Reaction time is critical. If reaction time exceeds two (2) minutes, drying of the reaction mixture may cause false positive results.
2. Freezing the CRP Latex Reagent will result in spontaneous agglutination.
3. Intensity of agglutination is not necessarily indicative of relative CRP concentration; therefore, screening reactions should not be graded.
4. A false negative can be attributed to a prozone phenomenon (antigen excess). It is recommended, therefore, to check all negative sera by retesting at a 1:10 dilution with glycine buffer.

### REFERENCE VALUES

Up to the reagent sensitivity (Indicated on the label of the latex vial). Each laboratory should establish its own reference range.

### PERFORMANCE CHARACTERISTICS

- **Sensitivity:** Refer to vial label.
- **Prozone effect:** No prozone effect was detected up to 1600 mg/L
- **Diagnostic sensitivity:** 95.6 %.
- **Diagnostic specificity:** 96.2 %.

### REFERENCES


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PPI2327A01
Rev A (05.01.2023)


| | | | |
|---|---|---|---|
| REF | Catalogue Number | | Temperature limit |
| IVD | *In Vitro* diagnostic medical device | | Caution |
| Σ | Contains sufficient for <n> tests and Relative size | | Consult instructions for use (IFU) |
| LOT | Batch code | | Manufacturer |
| | Fragile, handle with care | | Use-by date |
| | Manufacturer fax number | | Do not use if package is damaged |
| | Manufacturer telephone number | | Date of Manufacture |
| | Keep away from sunlight | | Keep dry |
| CONTROL+ | Positive control | CONTROL- | Negative control |

Atlas Medical CE 0459

# Blood Grouping Reagents:
# Anti-A Monoclonal Reagent, Anti-B Monoclonal Reagent, Anti-AB Monoclonal Reagent, Anti-D IgG/IgM blend Reagent, & Their variants
# SLIDE AND TUBE TESTS

IVD **For *In-Vitro* and professional use only**

2℃ 8℃ **Store at 2- 8°C**

**INTENDED USE**

The blood grouping reagents are used to detect the presence or absence of A, B or Rhesus Antigens on the surface of human red blood cells based on hemaglutination using slide or tube test techniques in whole blood samples or anticoagulant blood samples collected in EDTA , citrate or heparin tubes.

**INTRODUCTION & PRINCIPLES**

Blood grouping reagents are prepared from In-Vitro culture supernatants of hybridized immunoglobulin-secreting mouse cell lines. The reagents are diluted with phosphate buffer containing sodium chloride, EDTA and bovine albumin to give reagents that are optimized for use in tube and slide procedures. **Anti-A monoclonal reagent is colored with acid blue (patent blue) dye, Anti-B monoclonal reagent is colored with acid yellow (tartrazine) dye, and Anti-AB monoclonal reagent is not colored.** The test procedure is based on hemaglutination principle, where red cells possessing the antigen agglutinate in the presence of the corresponding antibody indicating that the result is positive. The test is considered negative when no agglutination appears.

Anti-D IgG/IgM blend reagent is prepared from carefully blended human monoclonal IgM and IgG. Anti-D IgG/IgM blend reagent is suitable for slide and tube test procedures. The reagent will directly agglutinate Rh D positive cells, including majority of variants (but not $D^{VI}$) and a high proportion of weak D (Du) phenotypes. The reagent will agglutinate category $D^{VI}$ and low grade weak D ($D^{u}$) phenotypes by the indirect anti-globulin techniques.

Anti-D IgG/IgM blend reagent is diluted with a sodium chloride solution, sodium phosphate solution and bovine albumin (sodium caprylate free). Anti-D IgG/IgM blend reagent is not colored. The procedure is based on hemaglutination principle, where red cells' possessing the antigen agglutinates in the presence of the corresponding antibody in the reagent indicating that the result is positive. The test is considered negative when no agglutination appears.

**MATERIALS**

**MATERIALS PROVIDED**

Blood Grouping Reagents:

- Anti-A monoclonal reagent (10 ml/vial), Clone: (9113D10).
- Anti-B monoclonal reagent (10 ml/vial), Clone: (9621A8).
- Anti-AB monoclonal reagent (10ml/vial), Clone: (152D12+9113D10).
- Anti-D IgG/IgM Blend reagent (10 ml/vial), Clone: (P3X61 + P3X21223B10 + P3X290 + P3X35).

**MATERIALS NEEDED BUT NOT PROVIDED**

- Plastic test tube or glass.
- Isotonic saline solution (% 0.9) NaCl).
- Applicator sticks.
- Centrifuge (100-1200 (g) for tube test).
- Timer.
- Incubator
- Anti-Human Globulin Reagent (can be ordered from Atlas Medical).
- White or transparent glass slide.

**PRECAUTIONS**

- The reagents are intended for in vitro diagnostic use only.
- The test is for well trained professional healthy user not for lay user.
- These reagents are derived from animal and human sources, thus, appropriate care must be taken in the use and disposal of these reagents, as there are no known test methods that can guarantee absence of infectious agents.
- Do not use reagents if it is turbid or contain particles as this may indicate reagent deterioration or contamination.
- Protective clothing should be worn when handling the reagents.
- **The reagents contain (0.1-0.2%) Sodium Azide and 0.02% sodium arseniate which is toxic and can be absorbed through the skin. When drained, the drains should be thoroughly flushed with water.**
- The reagents should be used as supplied and in accordance to the procedure mentioned below. Don't use beyond expiration date.
- Avoid cross contamination of reagents or specimens.
- Visible signs of microbial growth in any reagent may indicate degradation and the use of such reagent should be discontinued.
- Don't use these reagents if the label is not available or damaged.
- Do not use dark glass slide.
- Don't use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.
- Wash hands and the test table top with water and soap once the testing is done.
- Heamolysed blood sample should not be used for testing.
- The test should be performed at room temperature in a well let area with very good visibility.
- Failure to follow the procedure in this package insert may give false results or safety hazard.
- Close the vial tightly after each test.
- The reagent is considered toxic, so don't drink or eat beside it.
- If spillage of reagent occurs clean with disinfectant (disinfectant used could be irritable so handle with care).

**STORAGE CONDITIONS**

- The reagents should be stored refrigerated between 2 - 8°C.
- Never Freeze or expose to elevated temperature.
- The reagent is stable until the expiry date stated on the product label. Do not use the reagents past the expiry date.

**REAGENT PREPRATION**

- The reagents are intended for use as supplied, no prior preparation or dilution of the reagent is required.
- All reagents should be brought to room temperature before use.

**SPECIMEN COLLECTION AND PREPARATION**

- Blood collected with or without anticoagulant (EDTA, Heparin or Citrate) can be used for Antigen typing.

**Note:** Blood collected without anticoagulant should be tested immediately.

- The specimens should be tested as soon as possible after collection. If testing is delayed, the specimens should be stored at 2- 8 ºC, Sample must be retained to room temperature prior to analysis. (Testing should be carried out within five days of collections).
- Insure that there is no sign of hemolysis.
- At the time of the test, centrifuge the blood sample at 1200 RCF for 3 minutes.
- Blood collection is to be done with great care.

**PROCEDURES**

**A. DIRECT TUBE METHOD AT ROOM TEMPERATURE**

1. Prepare a 5% suspension of red blood cells in isotonic solution.
2. Using the vial dropper, transfer a drop (40±10µl) of each reagent into a separate and appropriately marked tube.
3. Add 50 µl of red blood cell suspension prepared in step 1.
4. Shake to homogenize the mixture, then centrifuge at 500g for **1 minute.**
5. Gently shake the tube in such a way to detach the cell pellet and macroscopically observe for any possible agglutination.
6. Read the reaction immediately.
7. For Anti-D tube, if the reaction is weak or negative, shake the tubes and incubate at 37°C for **15 minutes.**
8. Wash the red blood cells twice with isotonic saline solution (NaCl 0.9%) and discard the last washing liquid.
9. Add one drop (50µl) of the AHG reagent into the tube. Mix and centrifuge at 120g for **1 minute.**
10. Gently shake the tube in such a way to detach the cell pellet and macroscopically observe for any possible agglutination.
11. Read the reaction immediately.

**B. ANTIGLOBULIN INDIRECT METHOD for ANTI-D**

1. After immediately centrifuging and reading as above, if the reaction is weak or negative, shake the tubes and incubate at 37°C for 15 minutes.
2. Wash the red blood cells twice with isotonic saline solution (NaCl 0.9%) and discard the last washing liquid.
3. Add one drop (40 µl ± 10 µl) of ANTI-HUMAN GLOBULIN to the tube. Mix and centrifuge at 120 (g) for **1 minute**.
4. Gently shake the tube in such a way to detach the cell pellet and macroscopically observe for any possible agglutination.
5. Read the reaction immediately.

**C. DIRECT SLIDE METHOD AT ROOM TEMPERATURE**

1. Bring reagents and samples to room temperature (18-25°C).
2. Using the wax pen divide the slide into appropriate numbers of divisions.
3. Using the provided dropper, place one drop (40 µl ± 10 µl) of each reagent onto its correspondent division on the slide.
4. Add 25µl of the precipitated cells next to each drop of reagents.
5. Mix the reagent and the cells using a clean stirring stick over an area with a diameter of approximately 20-40mm.
6. Incubate the slide at room temperature (18-25°C) without stirring for **30 seconds**.
7. Hold the slide and gently rock the slide for **3 minutes** and observe macroscopically for any agglutination.
8. Read the reaction immediately.

**READING THE RESULT**

**POSITIVE:** If Agglutination appears.

**NEGATIVE:** If no agglutination is observed.

**Use the below table to determine the blood group:**

| Result of each reaction | | | | ABO Group |
|---|---|---|---|---|
| Anti-A monoclonal reagent | Anti-B monoclonal reagent | Anti-AB monoclonal reagent | Anti-D IgG/IgM blend reagent | |
| + | - | + | + | **A+** |
| + | - | + | - | **A-** |
| - | + | + | + | **B+** |
| - | + | + | - | **B-** |
| + | + | + | + | **AB+** |
| + | + | + | - | **AB-** |
| - | - | - | + | **O+** |
| - | - | - | - | **O-** |

**STABILITY OF THE REACTIONS**

- ABO Blood Grouping Tube tests should be read immediately following centrifugation.
- Slide tests should be interpreted within three minutes to avoid the possibility that a negative result may be incorrectly interpreted as positive due to drying of reagents.
- Delay in reading and interpreting results may result in weekly positive or falsely negative reactions. Slide tests should be interpreted at the end of the three minutes.

**PROCEDURE LIMITATION**

1. False positive/ negative results may occur due to:
   - Contamination from test materials.
   - Improper storage, cells concentration, incubation time or temperature.
   - Improper or excessive centrifugation.
   - Deviation from the recommended technique.
   - Blood samples of weak A or B subgroups may give rise to false negative results or weak reactions when tested using slide test method. It is advisable to re-test weak subgroups using tube test method.
2. Weaker reactions may be observed with stored blood than with fresh blood.
3. ABO antigens are not fully developed at birth, weaker reactions may therefore occur with cord or neonatal red cells.
4. ABO blood grouping interpretation on individuals greater than 6 months old should be confirmed by testing serum or plasma of the individual against group A and group B red cells (reverse grouping). If the results obtained with the serum do not correlate with the red cell test, further investigation is required.
5. Return the kit to the agent if it does not function properly.
6. Anti-D IgG/IgM blend Reagent tests conducted on particular weak-D phenotypes, while satisfactory, cannot ensure recognition of all weak variants, due to the variability of antigen patterns.

**DIAGNOSTIC PERFORMANCE CHARACTERISTICS**

The following tables compare the results in slide and tube techniques of 3 lots of Atlas Medical reagents and the results of a CE marked device.

| Slide Technique | | | | |
|---|---|---|---|---|
| Group A | | | | |
| Positive with anti-A monoclonal reagent and anti-AB monoclonal reagent<br>Negative with anti-B and Negative control | | | | |
| CE marked device | Lot A | Lot B | Lot C | Compliance |
| 232 | 232 | 232 | 232 | 100% |
| Tube Technique | | | | |
| Group A | | | | |
| Positive with anti-A monoclonal reagent and anti-AB monoclonal reagent<br>Negative with anti-B and Negative control | | | | |
| CE marked device | Lot A | Lot B | Lot C | Compliance |
| 212 | 212 | 212 | 212 | 100% |

| Slide Technique | | | | |
|---|---|---|---|---|
| Group B | | | | |
| Positive with anti-B monoclonal reagent and anti-AB monoclonal reagent<br>Negative with anti-A and Negative control | | | | |
| CE marked device | Lot A | Lot B | Lot C | Compliance |
| 61 | 61 | 61 | 61 | 100% |
| Tube Technique | | | | |
| Group B | | | | |
| Positive with anti-B monoclonal reagent and anti-AB monoclonal reagent<br>Negative with anti-A and Negative control | | | | |
| CE marked device | Lot A | Lot B | Lot C | Compliance |
| 61 | 61 | 61 | 61 | 100% |

| Slide Technique | | | | |
|---|---|---|---|---|
| Group O | | | | |
| Negative with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent<br>Negative with Negative control | | | | |
| CE marked device | Lot A | Lot B | Lot C | Compliance |
| 241 | 241 | 241 | 241 | 100% |
| Tube Technique | | | | |
| Group O | | | | |
| Negative with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent<br>Negative with Negative control | | | | |
| CE marked device | Lot A | Lot B | Lot C | Compliance |
| 243 | 243 | 243 | 243 | 100% |

| Slide Technique | | | | |
|---|---|---|---|---|
| Group AB | | | | |
| Positive with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent<br>Negative with Negative control | | | | |
| CE marked device | Lot A | Lot B | Lot C | Compliance |
| 33 | 33 | 33 | 33 | 100% |
| Tube Technique | | | | |
| Group AB | | | | |
| Positive with anti-A monoclonal reagent, Anti-B monoclonal reagent and anti-AB monoclonal reagent<br>Negative with Negative control | | | | |
| CE marked device | Lot A | Lot B | Lot C | Compliance |
| 24 | 24 | 24 | 24 | 100% |

No inversion in diagnosis has been shown: from a qualitative point of view we have observed 100% compliance in direct group testing in slide and tube techniques for determination of A, B, AB and O groups for the three lots of Atlas Medical.

**QUALITY CONTROL**

The reactivity of all blood grouping reagents should be confirmed by testing known positive and negative red blood cells on each day of use.

To confirm the specificity and sensitivity, Blood grouping reagents should be tested with antigen-positive and antigen-negative red blood cells.

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**PPI861A01**
**Rev.L (19.02.2022)**

CE 0459

**LIST OF VARIENTS:**

| Product Code | Product Name |
|---|---|
| 8.02.00.0.0010 | Anti-A Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 1 vial/Carton Box |
| 8.02.00.1.0100 | Anti-A Monoclonal Reagent (Titer: 1 /512), 10ml/vial. 10 vials / Plastic Pack |
| 8.02.00.1.0180 | Anti-A Monoclonal Reagent (Titer: 1 /512), 10ml/vial. 18 vials / Carton Box |
| 8.02.01.0.0010 | Anti-B Monoclonal Reagent (Titer: 1 /512), 10ml/vial, / Carton Box |
| 8.02.01.1.0100 | Anti-B Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 10 vials / Plastic Pack |
| 8.02.01.1.0180 | Anti-B Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 18 vials / Carton Box |
| 8.02.02.0.0010 | Anti-AB Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 1 vial/ Carton Box |
| 8.02.02.1.0100 | Anti-AB Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 10 vials/Plastic Pack |
| 8.02.02.1.0180 | Anti-AB Monoclonal Reagent (Titer: 1 /512), 10ml/vial, 18 vials/Carton Box |
| 8.02.03.0.0010 | Anti-D IgG/IgM Blend Reagent (Titer: 1 /128), 10ml/vial, 1 vial/ Carton Box |
| 8.02.03.1.0100 | Anti-D IgG/IgM Blend Reagent (Titer: 1 /128), 10ml/vial, 10 vials / Plastic Pack |
| 8.02.03.1.0180 | Anti-D IgG/IgM Blend Reagent (Titer: 1 /128), 10ml/vial, 18 vials / Carton Box |
| 8.02.04.0.0010 | Anti-A Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 1 Vial/Carton Box |
| 8.02.04.0.0100 | Anti-A Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 10 vials / Plastic Pack |
| 8.02.05.0.0010 | Anti-B Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 1vial/Carton Box |
| 8.02.05.0.0100 | Anti-B Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 10 vials /Plastic Pack |
| 8.02.05.6.0030 | ABO Set (Anti-A (1/256), Anti-B (1 /256), Anti-D (1/64)),3x10ml / plastic Pack |
| 8.02.05.7.0020 | ABO Set: Anti-A (1/256), Anti-B (1 /256), 2x10ml /Plastic Pack |
| 8.02.06.0.0010 | Anti-AB Monoclonal Reagent (Titer: 1 /256), 10ml/vial, 1vial/Carton Box |
| 8.02.06.1.0100 | Anti-AB Monoclonal Reagent (Titer: 1 /256), 10ml/vial,10 vials /Plastic Pack |
| 8.02.06.1.0180 | Anti-AB Monoclonal Reagent (Titer: 1 /256), 10ml/vial,18 vials / Carton Box |
| 8.02.07.0.0010 | Anti-D IgG/IgM Blend Reagent (Titer: 1 /64), 10ml/vial, 1Vial/ Carton Box |
| 8.02.07.1.0100 | Anti-D IgG/IgM Blend Reagent (Titer: 1 /64), 10ml/vial, 10 vials / Plastic Pack |
| 8.02.47.0.0030 | ABO Set (Anti-A (1 /512), Anti-B (1 /512), Anti-D (1 /128)),3x10ml/Plastic Pack |
| 8.02.47.1.0030 | ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-D (1 /64)), 3x10ml /Carton Box. |
| 8.02.47.3.0030 | ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-D (1 /64)), 3x10ml /Plastic Pack |
| 8.02.47.5.0030 | ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-D (1 /128)), 3x10ml/Plastic Pack |
| 8.02.49.0.0040 | ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-AB (1 /256), Anti-D (1 /64)), 4x10ml/Carton Box |
| 8.02.49.2.0040 | ABO Set (Anti-A (1 /256), Anti-B (1 /256), Anti-AB (1 /256), Anti-D (1 /128)), 4 x 10ml, 4 vials/Plastic Pack |
| 8.02.53.0.0040 | ABO Set (Anti-A (1 /512), Anti-B (1 /512), Anti-AB (1 /512) Anti-D (1 /128)), 4x10ml/Plastic Pack |
| 8.02.53.1.0040 | ABO Set (Anti-A (1 /512), Anti-B (1 /512), Anti-AB (1 /512) Anti-D (1 /128)), 4x10ml, 4vials/Plastic Pack |
| 8.02.70.0.0010 | Anti-A monoclonal reagent , Titer (1/1024), 10 ml/vial, 1Vial/ Carton Box |
| 8.02.71.0.0010 | Anti-B Monoclonal reagent (Titer: 1 /1024) , 10 ml/vial ,1Vial/ Carton Box |
| 8.02.72.0.0010 | Anti-AB Monoclonal reagent (Titer: 1 /1024) , 10 ml/vial , 1Vial/ Carton Box |
| 8.02.85.0.0010 | Anti-D IgG/IgM Blend reagent ( Titer 1 /256), 10ml/vial, 1Vial/ Carton Box |

| | | | |
|---|---|---|---|
| REF | Catalogue Number | | Temperature limit |
| IVD | *In Vitro* diagnostic medical device | | Caution |
| | Contains sufficient for <n> tests and Relative size | | Consult instructions for use (IFU) |
| LOT | Batch code | | Manufacturer |
| | Fragile, handle with care | | Use-by date |
| | Manufacturer fax number | | Do not use if package is damaged |
| | Manufacturer telephone number | | Date of Manufacture |
| | Keep away from sunlight | | Keep dry |

# GRAM STAIN PACK

IVD **For *in -vitro* diagnostic and professional use only**

15°C 30°C **Store at Room Temperature**

**INTENDED USE**

Gram Stain used for differentiate between gram positive and gram-negative bacteria.

**INTRODUCTION**

Gram staining is used to differentiate bacterial species into two large groups (Gram-positive and Gram-negative) based on the physical properties of their cell walls.

**PRINCIPLE**

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall), which stains Blue while gram-negative bacteria have a thinner layer (10% of cell wall), which stains pink. Gram-negative bacteria also have an additional outer membrane which contains lipids, and is separated from the cell wall by the periplasmic space. There are four basic steps of the Gram stain, which include applying a primary stain (crystal violet) to a heat-fixed smear of a bacterial culture, followed by the addition of a trapping agent (Gram's iodine), rapid decolorization with alcohol or acetone, and *counterstaining* with safranin or basic fuchsin.

Crystal violet (CV) dissociates in aqueous solutions into $CV^+$ and chloride ($Cl^-$) ions. These ions penetrate through the cell wall and cell membrane of both gram-positive and gram-negative cells. The $CV^+$ ion interacts with negatively charged components of bacterial cells and stains the cells Blue.

Iodine ($I^-$ or $I_3^-$) interacts with $CV^+$ and forms large complexes of crystal violet and iodine (CV–I) within the inner and outer layers of the cell. Iodine is often referred to as a mordant, but is a trapping agent that prevents the removal of the CV-I complex and therefore color from the cell.

When a decolorizer such as alcohol or acetone is added, it interacts with the lipids of the cell membrane. A gram-negative cell will lose its outer membrane and the lipopolysaccharide layer is left exposed. The

CV–I complexes are washed from the gram-negative cell along with the outer membrane. In contrast, a gram-positive cell becomes dehydrated from an ethanol treatment. The large CV–I complexes become trapped within the gram-positive cell due to the multilayered nature of its peptidoglycan. The decolorization step is critical and must be timed correctly; the crystal violet stain will be removed from both gram-positive and negative cells if the decolorizing agent is left on too long (a matter of seconds).

After decolorization, the gram-positive cell remains Blue. and the gram-negative cell loses its Blue. color. Counterstain, which is usually positively charged safranin or basic fuchsin, is applied last to give decolorized gram-negative bacteria a pink or red color.

**MATERIALS**

**MATERIALS PROVIDED**

- Crystal Violet.
- Gram Iodine.
- Gram Decolouriser.
- Counterstain – Safranin O.

**Note: This package insert is also used for individually packed reagent.**

**Storage and stability**

- Store at room temperature.
- Stain Solution is stable up to the printed expiry date.
- Keep the bottles tightly closed to prevent air oxidation.

**Precautions**

- The reagent may cause eye, skin and respiratory tract irritation; so protective clothing should be worn when handling this reagent.
- The reagent is intended for in vitro diagnostic use only.
- Do not use this reagent if the label is not available or damaged.
- Test materials and samples should be discarded properly in biohazards container.
- This reagent is considered toxic, so do not drink or eat beside it.
- Wash hands and test table top with water and soap once the testing is done.

**PROCEDURE**

1. immerse the heat fixed smears with Crystal Violet and allow to stain for up to 1 minute.
2. Wash with tap water.
3. Flood the smear with Gram Iodine for 2 minutes.
4. Wash with tap water.
5. Decolorize the smear for few second only.
6. Wash thoroughly with tap water.
7. Counterstain with Safranin O for up to 2 minutes.
8. Wash and allow to dry.
9. Examine under microscope using oil immersion objective

**RESULTS**

- Gram positive organisms (Blue).
- Gram negative organisms (Red).

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**PPI2112A01**
**Rev B (08.10.2020)**

| | | | |
|---|---|---|---|
| REF | Catalogue Number | | Temperature limit |
| IVD | *In Vitro* diagnostic medical device | | Caution |
| Σ | Contains sufficient for <n> tests and Relative size | | Consult instructions for use (IFU) |
| LOT | Batch code | | Manufacturer |
| | Fragile, handle with care | | Use-by date |
| | Manufacturer fax number | | Do not use if package is damaged |
| | Manufacturer telephone number | | Date of Manufacture |
| | Keep away from sunlight | | Keep dry |
| | Flammable | | |

Atlas Medical

## RF LATEX KIT

IVD For In-*Vitro* diagnostic and professional use only

Store at 2-8°C CE

**INTENDED USE**

Atlas RF latex test for the qualitative and semi-quantitative measurement of RF in human serum.

**INTRODUCTION**

Rheumatoid factors (RF) are antibodies directed against antigenic sites in the Fc fragment of human and animal IgG. Their frequent occurrence in rheumatoid arthritis makes them useful for diagnosis and monitoring of the disease.

One method used for rheumatoid factor detection is based on the ability of rheumatoid arthritis sera to agglutinate sensitized sheep red cells, as observed by Waaler and Rose. A more sensitive reagent consisting of biologically inert latex beads coated with human gamma globulin was later described by Singer and Plotz. The RF kit is based on the principle of the latex agglutination assay of Singer and Plotz. The major advantage of this method is rapid performance (2-minutes reaction time) and lack of heterophile antibody interference.

**PRINCIPLE**

The RF reagent is based on an immunological reaction between human IgG bound to biologically inert latex particles and rheumatoid factors in the test specimen. When serum containing rheumatoid factors is mixed with the latex reagent, visible agglutination occurs.

**MATERIALS**

**MATERIALS PROVIDED**

- RF Latex Reagent: Latex particles coated with human gamma-globulin, pH, 8,2. Preservative.
- RF Positive Control Serum (Red Cap): Human serum with a RF concentration > 30 IU/Ml. Preservative.
- RF Negative Control Serum (Blue Cap): Animal serum. Preservative.
- Glass Slide
- Stirring sticks

**NOTE: This package insert is also used for individually packed reagent.**

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Mechanical rotator with adjustable speed at 80-100 r.p.m.
- Vortex mixer.
- Pippetes 50 µL
- Glycine Buffer 20x (1000mmol/L): add one part to nineteen parts of distilled water before use.

**Packaging contents**

REF **8.00.04.0.0100 (1x4ml Latex Reagent, 1x1ml positive control, 1x1ml negative control)**

**PRECAUTIONS**

- All reagents contain 0.1 %(w/v) sodium azide as a preservative.
- Protective clothing should be worn when handling the reagents.
- Wash hands and the test table top with water and soap once the testing is done.
- Reagents containing sodium azide may be combined with copper and lead plumbing to form highly explosive metal azides. Dispose of reagents by flushing with large amounts of water to prevent azide buildup.
- For In Vitro diagnostic use.
- Components prepared using human serum found negative for hepatitis B surface antigen (HBsAg), HCV and antibody to HIV (1/2) by FDA required test. However, handle controls as if potentially infectious.
- Accuracy of the test depends on the drop size of the latex reagent (40µl). Use only the dropper supplied with latex and hold it perpendicularly when dispensing.
- Use a clean pipette tip and stirring stick for each specimen, and glass slides should be thoroughly rinsed with water and wiped with lint-free tissue after each use.
- Check reactivity of the reagent using the controls provided.
- Do not use these reagents if the label is not available or damaged.
- Do not use the kit if damaged or the glass vials are broken or leaking and discard the contents immediately.
- Test materials and samples should be discarded properly in a biohazard container.

**REAGENT PREPARATION:**

- The RF Latex reagent is ready to use. No preparation is required. Mix gently before use to ensure a uniform suspension of particles.

**STORAGE AND STABILITY**

- Reagents are stable until specified expiry date on bottle label when stored refrigerated (2-8°C).
- Do not freeze.
- Always keep vials in vertical position. If the position is changed, gently mix to dissolve aggregates that may be present.
- The RF latex reagent, once shaken must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal.
- Do not use the latex reagent or controls if they become contaminated.
- Reagents deterioration: Presence of particles and turbidity.

**SPECIMEN COLLECTION AND STORAGE**

- Use fresh serum collected by centrifuging clotted blood.
- If the test cannot be carried out on the same day, store the specimen for 7 days at 2-8°C and for 3 months at -20°C.
- Samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolyzed or lipemic samples.
- Do not use PLASMA.

**PROCEDURE**

**Qualitative method**

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.
2. Place (40 µL) of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Mix the RF-latex reagent rigorously or on a vortex mixer before using and add one drop (40 µL) next to the sample to be tested.
4. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample.
5. Place the slide on a mechanical rotator at 80-100 r.p.m. for 2 minutes. False positive results could appear if the test is read later than two minutes.

**Semi-quantitative method**

1. Make serial two-fold dilutions of the sample in 9 g/L saline solution.
2. Proceed for each dilution as in the qualitative method.

**READING AND INTERPRETATION**

Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide from the rotator. The presence of agglutination indicates a RF concentration equal or greater than 8 IU/mL (Note 1).

The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result.

**CALCULATIONS**

The approximate RF concentration in the patient sample is calculated as follows:

8 x RF Titer = IU/mL

**INTERFERENCES**

**NON-INTERFERING SUBSTANCES:**

- Hemoglobin (10g/L)
- Bilirubin (20mg/dl)
- Lipids (10g/L)

Other substances may interfere.

**QUALITY CONTROL**

- Positive and Negative controls are recommended to monitor the performance of the procedure, as well as a comparative pattern for a better result interpretation.
- All result different from the negative control result, will be considered as a positive.

**PERFORMANCE CHARACTERISTICS**

***Analytical sensitivity***

8 (6-16) IU/ml, under the described assay conditions.

***PROZONE EFFECT***

No prozone effect was detected up to 1500 IU/ml.

***DIAGNOSTIC SENSITIVITY***

100%.

***DIAGNOSTIC SPECIFICITY***

100%.

The diagnostic sensitivity and specificity have been obtained using 139 samples compared with the same method of a competitor.

**LIMITATIONS**

- Reaction time is critical. If reaction time exceeds 2 minutes, drying of the reaction mixture may cause false positive result.
- Freezing the RF Latex Reagent will result in spontaneous agglutination.
- Intensity of agglutination is not necessarily indicative of relative RF concentration; therefore, screening reactions should not be graded.
- Increased levels of RF may be found in some diseases other than rheumatoid arthritis such as infectious mononucleosis, sarcoidosis, lupus erythematosus, Sjogren's syndrome.
- Certain patients with rheumatoid arthritis will not have the RF present in their serum.
- The incidence of false positive results is about 3-5 %. Individuals suffering from infectious mononucleosis, hepatitis, syphilis as well as elderly people may give positive results.
- Diagnosis should not be solely based on the results of latex method but also should be complemented with a Waaler Rose test along with the clinical examination.

**REFERENCE VALUES**

Up to 8 IU/mL. Each laboratory should establish its own reference range.

**NOTES**

1. Results obtained with a latex method do not compare with those obtained with Waaler Rose test. Differences in the results between methods do not reflect differences in the ability to detect rheumatoid factors.

**REFERENCES**


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**PPI2326A01**
**Rev A (05.01.2023)**


| | | | |
|---|---|---|---|
| REF | Catalogue Number | | Temperature limit |
| IVD | *In Vitro* diagnostic medical device | | Caution |
| | Contains sufficient for <n> tests and Relative size | | Consult instructions for use (IFU) |
| LOT | Batch code | | Manufacturer |
| | Fragile, handle with care | | Use-by date |
| | Manufacturer fax number | | Do not use if package is damaged |
| | Manufacturer telephone number | | Date of Manufacture |
| | Keep away from sunlight | | Keep dry |
| CONTROL+ | Positive control | CONTROL- | Negative control |

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# ПАСПОРТ

## Масло иммерсионное, тип А (классическое), 100 мл

Серия **454/16** Дата выпуска **01.2022** Годен до **01.2025**
Количество флаконов в серии **20000**

| Наименование показателя | Требования по ГОСТ 13739-78 | Результаты анализа |
|---|---|---|
| **1. Внешний вид** | Жидкость от бесцветного до светло-желтого цвета | соответствует |
| **2. Технические характеристики** | | |
| 2.1. Вязкость кинематическая (ν), при 20 °С, м2/с*10-4, не менее | 6 | 13 |
| 2.2. Коэффициент пропускания (Т), при толщине слоя 1 мм, % | | |
| при длине волны 635 нм, не менее | 95 | 96 |
| при длине волны 440 нм, не менее | 92 | 98 |
| 2.3. Коэффициент преломления (n), при 20 °С | 1,515 ± 0,001 | 1,515 |
| 2.4. Средняя дисперсия (nf-nc) , при 20 °С | 0,0106 +/- 0,0003 | 0,0107 |

Заключение ОКК ООО «Агат-Мед»:
Набор серии **454/16** требованиям ГОСТ 13739-78 соответствует.

Начальник ОКК ООО «АГАТ-МЕД» Гладун В.В.

« 01 » января 2022 г.


МП

www.dialab.at

# STATEMENT

We, **DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.**, having a registered office at **IZ-NOE Sued Hondastrasse, Objekt M55, A-2351 Wr. Neudorf, AUSTRIA** assign SRL SANMEDICO having a registered office at A. Corobceanu street 7A, apt. 9, Chişinău MD-2012, Moldova , as authorized representative in correspondence with the conditions of directive 98/79/EEC. We declare that the company mentioned above is authorized to register, notify, renew or modify the registration of medical devices on the territory of the Republic of Moldova. This declaration will stay in force for 2 years or if one of the parties is deciding to cancel it with a one-month notice.

Date :05.04.2023
Signature:

DIALAB
Produktion und Vertrieb von chemisch - technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
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Christina Ernst
Export Manager

# Certificate of Analysis / Quality Control

**Product:** HBcAb

**REF:** Z00364 / Z00364V / Z00364LV

**Lot:** 0322/K20230301

**Exp.:** 2024-04-01

**Results:**

| Quality Control Panels | Acceptance Criteria | Test Results |
|---|---|---|
| 15 Positive Control Human Sera Samples | (+/+) ≥ 14/15 | (+/+) 15/15 |
| 15 Negative Control Human Sera Samples | (-/-) 15/15 | (-/-) 15/15 |
| Limit Of Detection: | | |
| 3 serial diluted samples (S1 - S3) | ≥ 2/3 | 2/3 |
| Precision: | | |
| Inter assay - 10 positive replicates | CV% ≤ 20 | CV%=10.22 |
| **Accelerated Stability Study** | **Acceptance Criteria** | **Test Results** |
| 15 Positive Control Human Sera Samples | (+/+) ≥ 14/15 | (+/+) 15/15 |
| 15 Negative Control Human Sera Samples | (-/-) 15/15 | (-/-) 15/15 |
| Limit Of Detection: | | |
| 3 serial diluted samples (S1 - S3) | ≥ 2/3 | 2/3 |
| Precision: | | |
| Inter assay - 10 positive replicates | CV% ≤ 20 | CV%=9.38 |

This lot meets our specifications.

Lucija Dilber 2023.05.09 16:43:16 +02'00'

Rev. 4

Date: 2018-03-28

www.dialab.at

**ELISA E**NZYME **L**INKED **I**MMUNO**S**ORBENT **A**SSAY

**Microwell Method**

# HBcAb

**REF Z00364**

*For in vitro Diagnostic Use*

P r o d u c t  I n s e r t

Enzyme Linked Immunosorbent Assay for the **cut-off** determination of antibodies to Hepatitis B core antigen (HBcAg) in human serum or plasma.

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Microwell Method - 96 wells

(12 x 8-well antigen coated strips

Individual breakaway)

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## INTRODUCTION

Hepatitis B core Antigen (or HBcAg) is the major component of the core particles of Hepatitis B virus (or HBV). Particles have a size of 27 nm and contain a circular double-stranded DNA molecule, a specific DNA-polymerase and HBeAg; HBcAg is composed of a single polypeptide of about 17 kD that is released upon dis-aggregation of the core particles; the antigen contains at least one immunological determinant. Upon primary infection, anti HBcAg antibodies are one of the first markers of HBV hepatitis appearing in the serum of the patient, together or slightly later than HBsAg, the viral surface antigen. Anti HBcAg antibodies are produced usually at high titers and their presence is detectable even years after infection. Isolated HBcAb, in absence of other HBV markers, have been observed in blood units, suggesting the use of this test for screening HBV, in addition of HBsAg. The determination of HBcAb has become important for the classification of the viral agent, together with the detection of the other markers of HBV infection, in sera and plasma.

## PRINCIPLE OF THE ASSAY

This anti-HBc ELISA kit is based on solid phase, one step incubation competitive principle ELISA. Anti-HBc if present in the sample competes with monoclonal anti-HBc conjugated to horseradish peroxidase (HRP) for a fixed amount of purified HBcAg pre-coated in the wells. When no anti-HBc is present in the sample, the HRP labeled anti-HBc will be bound with the antigens inside the wells and any unbound HRP-Conjugate is removed during washing. Chromogen B and A solutions are added into the wells and during incubation the colorless Chromogens are hydrolyzed by the bound HRP-Conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. No or low color developing suggests the presence of antibodies to HBcAg in the sample.

**Assay principle scheme: Competition ELISA**

| | | | | | | |
|---|---|---|---|---|---|---|
| **Ag(p)** | **+ Ab(s)+(Ab)ENZ** | **→ [Ag(p)–Ab(s)]** | **→** | | **No color** | **(+)** |
| **Ag(p** | **+ (Ab)ENZ** | **→ [Ag(p) – (Ab)ENZ ]** | **→** | **Blue →** | **Yellow Color** | **(- )** |
| **Incubation** | | **Immobilized Complex** | | | **Coloring** | **Results** |
| **60 min.** | | | | **15 min.** | | |

**Ag(p)–**pre-coated HBcAg;
**Ab(s)–** anti-HBc in sample;
**(Ab)ENZ**– HRP conjugated anti-HBc ;

## TEST CONDITIONS AND NOTICES

1. All the reagents contained in the kit are for “in vitro” diagnostic use only.
2. Do not use the kit or reagents after the expiry date stated on labels. Do not mix reagents of different lots.
3. Procedures should be performed carefully in order to obtain reliable results and clinical interpretations.
4. Bring all the reagents to room temperature for at least 60 min, before the test is started.
5. Avoid any contamination of reagents when taking them out of vials. We recommend use of automatic pipettes and disposable tips. When dispensing reagents, do not touch the wall of microplate wells with tips, in order to avoid any cross-contamination.
6. In the washing procedure, use only the Wash Buffer provided with the kit and follow carefully the indications reported in the “WASHING INSTRUCTIONS” section of this insert.

7. Ensure that the Substrate A/B mixture does not come in contact with oxidizing agents or metallic surfaces; avoid any intense light exposure during the incubation step or the reagent preparation.
8. Samples and materials potentially infective have to be handled with care as they could transmit infection.

   All objects come in direct contact with samples and all residuals of the assay should be treated or wasted as potentially infective. Best procedures for inactivation are treatments with autoclave at 121°C for 30 min or with sodium hypochlorite at a final concentration of 2.5% for 24 hrs. This last method can be used for the treatment of the liquid waste after that it has been neutralized with NaOH.
9. Avoid any contact of liquids with skin and mucosas.
   Use always protective talk-free gloves, glasses and laboratory coats, according to the safety regulations.

**CONTENT OF THE KIT**

| | |
|---|---|
| **Microwell Plate** | Blank microwell strips fixed on a white strip holder. The plate is sealed in aluminum pouch with desiccant. 12×8-well strips per plate. Each well contains purified HBcAg. The microwell strips can be broken to be used separately. Place unused wells in the plastic sealable storage bag together with the desiccant and return to 2-8°C. |
| **Enzyme Conjugate** | 6.5 mL per vial. Horseradish peroxidase-conjugated anti-HBc. Ready to use as supplied. Once open, stable for one month at 2-8°C. |
| **Wash Buffer** | 30 mL per bottle, pH 7.4, 20x PBS (containing Tween-20 as a detergent). The concentrate must be diluted 1:20 with distilled or deionized water before use. Once diluted, stable for one week at room temperature or for two weeks at 2-8°C. **DILUTE BEFORE USE!** |
| **Substrate Solution A** | 7 mL per vial. Urea peroxide solution. Ready to use as supplied. Once open, stable for one month at 2-8°C. |
| **Substrate Solution B** | 7 mL per vial. TMB solution- Tetramethylbenzidine dissolved in citric acid. Ready to use as supplied. Once open, stable for one month at 2-8°C. |
| **Stop Solution** | 7 mL per bottle. Diluted sulfuric acid solution (0.5 M $H_2SO_4$). Ready to use as supplied. |
| **Negative Control** | 1 mL per vial. Protein-stabilized buffer tested non-reactive for anti-HBc. Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8°C. |
| **Positive Control** | 1 mL per vial. Purified anti-HBc diluted in Protein stabilized buffer Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8°C. |
| **Cardboard Sealer** | 1 piece. To cover the plates during incubation and to prevent the well from evaporation or contamination. |

## STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, do not freeze. To assure maximum performance of this anti-HBc ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

## MATERIALS NOT PROVIDED

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Disposable V-shaped troughs.
5. Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
6. Absorbent tissue or clean towel.
7. Dry incubator or water bath, 37±0.5°C.
8. Microshaker for dissolving and mixing conjugate with samples.
9. Microwell plate reader, single wavelength 450 nm or dual wavelength 450 nm and 630 nm.
10. Microwell aspiration/wash system.

## SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

1. Sample Collection: Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM (rounds per minute) for 20 minutes at room temperature or by filtration on 0.22 µm filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolysed samples should not be used as they can give false results in the assay. Do not heat inactivate samples. This can cause sample deterioration.
2. Transportation and Storage: Store samples at 2-8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower). Avoid multiple freeze-thaw cycles.

## SPECIAL INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles of 350-400 µL/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with sample or Enzyme Conjugate, after incubation do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer liquid dispensing channels are not blocked or contaminated and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to carry out at least 5 cycles, dispensing 350-400 µL/well and aspirating the liquid for 5times. If poor results

(high background) are observed, increase the washing cycles or soaking time per well.

6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before liquids are wasted in an appropriate way.
7. The concentrated Washing solution should be diluted 1:20 before use. For one plate, mix 30 mL of the concentrate with 570 mL of water for a final volume of 600 mL diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

## PRECAUTIONS AND SAFETY

This kit is intended FOR PROFESSIONAL IN VITRO USE ONLY

**The ELISA assay is time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.**

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond the expiry date stated on labels or boxes.
3. Allow the reagents and samples to reach room temperature (18-30°C) before use. Shake reagent gently before use and return to 2-8°C immediately after use.
4. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
5. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.
7. Avoid assay steps long time interruptions. Assure same working conditions for all the wells.
8. Calibrate the pipette frequently to assure the accuracy. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations. Never pipette solutions by mouth.
9. The use of automatic pipettes and disposable tips is recommended.
10. Assure that the incubation temperature is 37°C inside the incubator.
11. When adding samples avoid touching the well's bottom with the pipette tip.
12. When reading the absorbance with a plate reader, it is recommended to determine the absorbance at 450 nm or at 450 nm with reference at 630 nm.
13. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke or apply cosmetics in the assay laboratory.
14. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1 hour at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps for disposal.

15. The Stop Solution contains 0.5 M $H_2SO_4$. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
16. The enzymatic activity of the Enzyme Conjugate might be affected from dust, reactive chemical and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.

## ASSAY PROCEDURE

Step 1 Reagents preparation: Allow the reagents to reach room temperature (18-30°C). Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the stock Wash Buffer 1:20 with distilled or deionized water. Use only clean vessels to dilute the buffer.

Step 2 Numbering Wells: Set the strips needed in strip-holder and number sufficient number of wells including three Negative Controls (e.g. B1, C1, D1) two Positive Controls (e.g. E1, F1) and one Blank (e.g. A1 neither samples nor Enzyme Conjugate should be added into the Blank well). Use only number of strips required for the test.

Step 3 Adding Sample and Enzyme Conjugate: Add 50 µL of Positive Control, Negative Control, and Specimen into their respective wells. Note: Use a separate disposal pipette tip for each specimen, Negative Control and Positive Control to avoid cross-contamination. Add 50 µL of Enzyme Conjugate to each well except the Blank and mix by tapping the plate gently.

Step 4 Incubating: Cover the plate with the plate cover and incubate for 60 minutes at 37°C. It is recommended to use water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

Step 5 Washing: At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the strip plate onto blotting paper or clean towel, and tap the plate to remove any remainders.

Step 6 Coloring: Dispense 50 µL of Substrate Solution A and after that 50 µL Substrate Solution B into each well including the Blank. Incubate the plate at 37°C for 15minutes, avoiding light. The enzymatic reaction between the Substrate Solutions and the Enzyme Conjugate will produce blue color in Negative Control and anti-HBc negative sample wells.

Step 7 Stopping Reaction: Using a multichannel pipette or manually add 50 µL Stop Solution into each well and mix gently. Intensive yellow color develops in Negative control and anti-HBc negative sample wells.

Step 8 Measuring the Absorbance: Calibrate the plate reader with the Blank well and read the absorbance at 450 nm. If a dual filter instrument is used, set the reference wavelength at 630 nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 5 minutes after stopping the reaction).

## INTERPRETATION OF RESULTS AND QUALITY CONTROL

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on Dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

**Calculation of cut-off value (C.O.) = *Nc × 0.5**
*Nc = the mean absorbance value for three negative controls.

If one of the Negative control values does not meet the Quality Control Range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

**Example:** of Cut-off calculation:
1. Calculation of Nc

| Well No: | B1 | C1 | D1 |
|---|---|---|---|
| Negative controls OD value | 1.720 | 1.715 | 1.717 |

Nc=1.717
2. Calculation of Cut-off (C.O.)= 1.729 × 0.5 =0.858

## QUALITY CONTROL RANGE

The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

1. The absorbance of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.

2. The absorbance value OD of the Negative control must be equal to or greater than 0.800 at 450/630 nm or at 450 nm after blanking.

3. The absorbance value OD of the Positive control must be less than 0.100 at 450/630 nm or at 450 nm after blanking .

## INTERPRETATIONS OF THE RESULTS

(S = the individual absorbance (OD) of each specimen)

**Negative Results (S/C.O.>1):**

Samples giving an absorbance greater than the Cut-off value are considered negative, which indicates that no antibodies to HBV core antigen have been detected using this anti-HBc ELISA kit. This result should not be used alone to establish the infection state.

**Positive Results (S/C.O.≤1):**

Samples giving absorbance less than or equal to the Cut-off value are initially reactive for this assay, which indicates that antibodies to HBV core antigen have probably been detected with this anti-HBc ELISA kit. Any initially reactive samples must be retested in duplicates. Repeatedly reactive samples can be considered positive for anti-HBc. A positive result with anti-HBc detection is an indication of acute HBV infection. Monitoring of anti-HBc concentrations can be used in follow up of chronic HBV patients. However, any positive result should not be used alone to establish the infection state.

**Borderline (S/C.O.=0.9-1.1):**

Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline samples and retesting is recommended. Repeatedly reactive samples can be considered positive for anti-HBc.

## TEST PERFORMANCE AND EXPECTED RESULTS

Analytical Endpoint Sensitivity: 0.8PEI U/mL

The **clinical specificity** of this assay has been determined by a panel of samples obtained from 1683 healthy blood donors and 145 undiagnosed hospitalized patients. The Repeatedly reactive samples and samples confirmed positive with the reference test were not included in the calculation of the specificity.

The **clinical sensitivity** of this anti-HBc ELISA kit have been calculated by a panel of samples obtained from 975 hepatitis B patients with well-characterized clinical history based upon reference assays for detection of HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc. This panel included samples from acute, chronic and recovered hepatitis B patients. Licensed anti-HBc ELISA test was used as a confirmatory assay. The evaluation results are given below. Results obtained in individual laboratories may differ.

| **Specificity** | Samples | - | + | Confirmed positive | **Specificity** | False Positive |
|---|---|---|---|---|---|---|
| Blood donors | 1683 | 566 | 1117 | 1115 | 99.64% | 2 |
| Hospitalized patients | 145 | 80 | 65 | 65 | 100% | 0 |
| **TOTAL** | **1828** | **646** | **1182** | **1180** | **99.82** | **2** |

| **Sensitivity** | Samples | - | + | Confirmed positive | **Sensitivity** | False Negative |
|---|---|---|---|---|---|---|
| Acute | 429 | 11 | 417 | 418 | 99.76% | 1 |
| Chronic | 105 | 0 | 105 | 105 | 100% | 0 |
| Recovery | 441 | 5 | 436 | 436 | 100% | 0 |
| **TOTAL** | **975** | **16** | **958** | **959** | **99.92** | **1** |

**Analytical Specificity:**

1. No cross reactivity observed with samples from patients infected with HAV, HCV HIV, CMV, and TP.
2. No interference from rheumatoid factors up to 2000U/mL observed during clinical testing.
3. The assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein.
4. Frozen specimens have been tested to check for interferences due to collection and storage.

| **Reproducibility** | No runs | **Within run** | | **Between run** | |
|---|---|---|---|---|---|
| | | Mean OD | CV% | Mean OD | CV% |
| Weak positive | 10 | 0.639 | 5.8% | 0.645 | 6.4% |
| Moderate positive | 10 | 0.394 | 7.4% | 0.404 | 8.0% |
| Strong positive | 10 | 0.012 | 21% | 0.017 | 22% |
| Negative control | 10 | 1.768 | 4.5% | 1.702 | 4.6% |

## LIMITATIONS

1. Non-repeatable positive result may occur due to the general biological and biochemical characteristics of ELISA assays. The test is designed to achieve very high performance characteristics of sensitivity and specificity. However, in very rare cases some HBV mutants or subtypes can remain undetectable. Antibodies may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. Any positive results must be interpreted in conjunction with patient clinical information and other laboratory testing results.
3. Common sources for mistakes: kits beyond the expiry date, inappropriate washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
4. The prevalence of the marker will affect the assay's predictive values.

## VALIDITY

**Please do not use this kit beyond the expiry date indicated on the kit box and reagent labels!**

## REFERENCES

1. Hansson, B.G. (1977). Persistence of Serum Antibody to Hepatitis B Core Antigen. J. Clin. Microbiol. 6, 209.
2. Hoofnagle, J.H., Gerety, R.J. and Barker, L.F. (1973). Antibody to Hepatitis B Virus Core in man. Lancet, 869.
3. Hoofnagle, J.H., Gerety, R.J., Ni, L.Y. and Barker, L.F. (1974).
4. Antibody to Hepatitis B Core Antigen. N. Engl. J. Med., 290, 1336
5. Mushahwar, I.K.,Dienstag,J .L.,Polesky,H.F et al (1981) Interpretation of Various Serological Profiles of Hepatitis B Virus Infection.Am J. Clin Pathol,76,773

**ELISA** **E**nzyme **L**inked **I**mmuno**s**orbent **A**ssay

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ZERTIFIKAT ◆ CERTIFICATE ◆ 認證證書 ◆ СЕРТИФИКАТ ◆ CERTIFICADO ◆ CERTIFICAT

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TÜV SÜD Product Service

# Certificate

## No. Q5 026709 0009 Rev. 01

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**Certification Mark:**


**Scope of Certificate:**

**Design, development, production and distribution of in-vitro diagnostic reagents and testkits in the areas of immunological detection of infectious diseases, immunochemistry/immunology/clinical chemistry biomarkers (analytes: enzymes, substrates, electrolytes reagents; controls/standards/calibrators), urinalysis, haematology, haemostasis and immunohaematology (blood grouping).**
**Distribution of in-vitro diagnostic instruments including accessories for immunology, clinical chemistry, haematology, haemostasis and urinalysis.**

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**Report No.:** 713237224

**Valid from:** 2022-03-29
**Valid until:** 2025-03-28

**Date,** 2022-03-17

Christoph Dicks
Head of Certification/Notified Body


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# Certificate

## No. Q5 026709 0009 Rev. 01

**Applied Standard(s):**

EN ISO 13485:2016
Medical devices - Quality management systems - Requirements for regulatory purposes
(ISO 13485:2016)
DIN EN ISO 13485:2016

**Facility(ies):**

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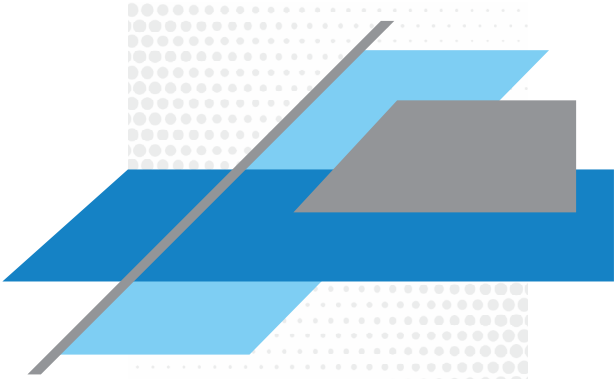
TÜV®

# anti-HCV

**ИФА-набор для качественного определения суммарных антител к вирусу гепатита С**

Инструкция по применению



IVD

REF
EI-021

96
анализов

UA.TR.061

# EQUI anti-HCV

ИФА-набор для качественного определения суммарных антител к вирусу гепатита С

## 1. НАЗНАЧЕНИЕ

ИФА-набор «EQUI anti-HCV» предназначен для качественного определения суммарных антител к вирусу гепатита С (ВГС) в сыворотке или плазме крови человека методом иммуноферментного анализа (ИФА) с целью диагностики гепатита С и скрининга донорской крови. Процедура анализа рассчитана как для ручной постановки с автоматическими пипетками и стандартным оборудованием, так и для автоматического иммуноферментного анализатора «открытого» типа.

**Целевая группа:** доноры; лица-потребители инъекционных наркотиков; реципиенты крови или органов; беременные женщины; дети, рожденные от инфицированных матерей; лица, инфицированные ВИЧ; пациенты с симптомами заболеваний печени.

**Применение:** ИФА-набор применяется в клинических диагностических лабораториях, станциях переливания крови, а также в других учреждениях, работающих в области *in vitro* диагностики.

## 2. КЛИНИЧЕСКОЕ ЗНАЧЕНИЕ

Гепатит С – это вирусное поражение печени. Вирус гепатита С (ВГС) вызывает острую инфекцию, которая может перейти в хроническую форму. Вирус гепатита С относится к семейству *Flaviviridae*. Вирионы маленькие (50-60 nm), сферические, покрытые оболочкой. Генетический материал вируса представлен одноцепочечной РНК. Передача вируса происходит гемотрансмиссивным путем, для заражения здорового организма достаточно небольшого количества крови, содержащей ВГС. Инкубационный период гепатита С может длиться от двух недель до полугода.

В связи с бессимптомным течением гепатит С редко выявляют на ранних стадиях. По рекомендациям международных организаций в сфере здравоохранения (ВОЗ, CDC) диагностика инфекции ВГС проводится в два этапа. Сначала проводится серологический скрининг на антитела к вирусу гепатита С. Затем для положительных образцов необходимо подтвердить наличие хронической гепатитной инфекции обнаружением РНК вируса. Антитела к ВГС при отсутствии генетического материала вируса не могут свидетельствовать об активной инфекции у пациента. После диагностирования гепатитной инфекции оценивают степень поражения печени (фиброза, цирроза), проводят дополнительные лабораторные тесты для назначения лечения и мониторинга его эффективности.

«Серологическое окно» и характер иммунного ответа после инфицирования могут сильно отличаться у разных пациентов. Первыми в крови инфицированных оказываются РНК и Core-антиген вируса (через 1-3 недели). ВГС-специфические антитела IgM класса обнаруживаются через 1-2 месяца после инфицирования. У 50-90% пациентов с острой гепатитной

инфекцией они могут определяться в высоких титрах несколько месяцев. Впоследствии, у 50-70% лиц, больных хроническим гепатитом С, IgM антитела обнаруживаются на невысоких уровнях, особенно при обострении инфекционного процесса. После эффективной терапии ВГС-специфические IgM антитела исчезают через несколько месяцев. Этот маркер важен для мониторинга лечения, но не является информативным для скрининга гепатита С. Продуцирование антител ВГС на относительно низком уровне во время острой фазы начинается почти одновременно с антителами класса IgM, но постепенно возрастает при хронизации инфекции. IgG антитела к ВГС производятся на высоких уровнях во время хронического гепатита С и могут проявляться даже после выведения вируса из организма.

При производстве иммуноферментных тестов третьего поколения для диагностики гепатита С используются рекомбинантные антигены ВГС, аналоги белков Core, NS3, NS4 и NS5. Чувствительность таких тестов достигает 99%, а специфичность приближается к 100%. Однако, чувствительность и специфичность ИФА тестов разных производителей могут несколько отличаться. Ложноотрицательные анализы встречаются у иммуносупрессированных лиц, а ложноположительные результаты могут быть связаны со способом получения рекомбинантных белков. Результаты обнаружения антител к ВГС необходимо подтверждать другими лабораторными методами.

## 3. ПРИНЦИП АНАЛИЗА

Определение антител, специфичных к ВГС, в ИФА-наборе «EQUI anti-HCV» базируется на принципе «непрямого» твердофазного ИФА в двухэтапной инкубации. В лунках планшета засорбированы рекомбинантные антигены вируса гепатита С: core, NS3, NS4 та NS5. При первом этапе инкубации исследуемых образцов в лунках планшета ИФА специфические к ВГС антитела, если они присутствуют в образцах, связываются с соответствующими антигенами на твердой фазе. Лунки отмываются для удаления несвязанных антител, остаются только специфические комплексы антиген-антитело. После этого добавляется смесь конъюгатов антивидовых (анти-IgG и анти-IgM) моноклональные антитела с пероксидазой хрена, которые связываются с иммунными комплексами на твердой фазе. Несвязанные компоненты удаляются при отмывании. Комплексы антиген-антитело обнаруживаются путем добавления раствора хромогена 3,3',5,5'-тетраметилбензидина (ТМБ) с перекисью водорода. После 30-минутной инкубации реакция останавливается добавлением стоп-раствора. Оптическая плотность (ОП) в лунках определяется на спектрофотометре при длине волны 450/620–695 nm. Интенсивность желтой окраски пропорциональна количеству антител в образце.

## 4. МАТЕРИАЛЫ И ОБОРУДОВАНИЕ

### 4.1. Состав набора

| | | |
|---|---|---|
| STRIPS | 1 x 96 лунок | **Планшет ИФА** В лунках планшета засорбированы рекомбинантные антигены ВГС: core, NS3, NS4 та NS5. Лунки можно отделять. После первого вскрытия храните неиспользованные стрипы в упаковке при температуре 2-8°C не более 6 месяцев |
| CONTROL + | 1 x 0,6 ml | **Позитивный контроль** Раствор иммуноглобулинов человека, специфичных к ВГС, с консервантом (розовый). Хранить при температуре 2-8°C |
| CONTROL - | 1 x 1,6 ml | **Негативный контроль** Негативная сыворотка крови человека с консервантом (желтый). Хранить при температуре 2-8°C |
| DIL SAMPLE | 1 x 11 ml | **Раствор для разведения сывороток** Буферный раствор с экстрактом молока, детергентом и консервантом (коричневый). Хранить при температуре 2-8°C |
| SOLN CONJ | 1 x 13 ml | **Раствор конъюгата (готов к использованию)** Буферный раствор моноклональных антител к IgG и IgM человека, конъюгированный с пероксидазой хрена, со стабилизаторами и консервантом (зеленый). Хранить при температуре 2-8°C |
| SOLN TMB | 1 x 13 ml | **Раствор ТМБ (готов к использованию)** Раствор ТМБ, H2O2, стабилизатор, консервант (бесцветный). Хранить при температуре 2-8°C |
| TRITON WASH 20x | 1 x 50 ml | **Раствор для промывки TRITON (20x концентрат)** 20-кратный концентрат фосфатного буфера с Тритоном X-100 (бесцветный). Развести раствор для промывки TRITON (20x) 1:20 дистиллированной или деионизированной водой (например, 5 ml концентрата + 95 ml воды для 8 лунок) перед использованием. Разбавленный раствор хранить при температуре 2-8°C не более 7 суток |
| SOLN STOP | 1 x 13 ml | **Стоп-раствор (готов к использованию)** Раствор 0,5 mol $H_2SO_4$ (бесцветный). Хранить при температуре 2-8°C |

В состав набора входят: клейкая пленка (2 шт.), схема внесения образцов (1 шт.), лист контрольных испытаний и инструкция по применению.

### 4.2. Дополнительные реактивы, материалы и оборудование

Автоматические пипетки переменного объема на 10–1000 µl и наконечники к ним, мерная лабораторная посуда (10–1000 ml), деионизированная или дистиллированная вода, термостат на 37°C, автоматический или полуавтоматический промыватель планшетов (вошер), спектрофотометр (ридер) для микропланшетов 450/620-695 nm, соответствующие контейнеры

для отходов потенциально зараженного материала, таймер, фильтровальная бумага, одноразовые неопудренные перчатки, дезинфицирующие средства.

## 5. ПРЕДОСТЕРЕЖЕНИЯ И ТЕХНИКА БЕЗОПАСНОСТИ

### 5.1. Предостережения

*Перед проведением анализа внимательно ознакомьтесь с инструкцией по применению. Достоверность результата зависит от четкого следования процедуре анализа.*

- не используйте компоненты ИФА-набора по истечении срока годности;
- не используйте при анализе и не смешивайте компоненты различных серий, компоненты из наборов различных нозологий или реагенты других производителей в сочетании с набором «EQUI anti-HCV»;
- не замораживайте ИФА-набор или его компоненты;
- после использования реагента закрывайте каждый флакон своей крышкой;
- во время промывки контролируйте наполнение и полную аспирацию раствора из лунок;
- каждый раз используйте новый наконечник пипетки для внесения образцов или реагентов;
- избегайте попадания прямых солнечных лучей на реагенты ИФА-набора;
- SOLN|TMB должно быть бесцветным перед использованием. Если раствор окрашен в синий или желтый цвет, его нельзя использовать. Избегайте контакта SOLN|TMB с металлами или ионами металлов. Для работы используйте только чистую, тщательно выполосканную дистиллированной водой посуду;
- не используйте реагенты, цвет которых не соответствует указанному в пункте 4.1;
- ни в коем случае не используйте одну и ту же посуду для SOLN|CONJ и SOLN|TMB;
- не проводите визуальный учет результатов анализа (без использования ридера);
- дополнительное оборудование, находящееся в непосредственном контакте с биологическим материалом или компонентами набора, считается загрязненным и требует очистки и обеззараживания;
- ИФА набор предназначен для 96 анализов. Компоненты после использования и остатки неиспользованных компонентов должны быть утилизированы.

### 5.2. Техника безопасности

- все реагенты набора предназначены только для профессионального лабораторного применения в *in vitro* диагностике и могут использоваться только квалифицированным персоналом;
- постановку анализа проводить только в одноразовых неопудренных перчатках и защитных очках;

– не допускается принимать пищу, пить, курить или пользоваться косметикой в комнате проведения теста;
– не пипетировать растворы ртом;
– CONTROL + ИФА-набора «EQUI anti-HCV» содержит иммуноглобулины человека, специфические к ВГС, которые были выделены с инактивированным прогреванием сывороток крови человека, в которых не было обнаружено HBsAg и антител к ВІЛ1/2 и *Treponema pallidum*, однако работать с контролем следует как с потенциально инфекционным материалом;
– CONTROL - ИФА-набора «EQUI anti-HCV» протестировано и признано отрицательным на HBsAg и антитела к ВІЛ1/2, ВГС, *Treponema pallidum*, однако обращаться с контролем и изучаемыми образцами следует как с потенциально опасным инфекционным материалом;
– некоторые компоненты набора содержат низкие концентрации вредных веществ и могут вызвать раздражение кожи и слизистых. При попадании SOLN TMB, SOLN STOP и SOLN CONJ на слизистые или кожу необходимо немедленно промыть пораженное место большим количеством воды;
– в случае разбрызгивания растворов, не содержащих кислоту, например сывороток, обработать поверхность дезинфицирующим средством, затем вытереть досуха фильтровальной бумагой. В противном случае кислоту сначала нужно нейтрализовать раствором бикарбоната натрия, а затем вытереть поверхность, как описано выше.

### 5.3. Инактивация и утилизация отходов

– жидкие отходы следует инактивировать, например, раствором перекиси водорода в конечной концентрации 6% в течение 3 часов при комнатной температуре или гипохлоритом натрия в конечной концентрации 5% в течение 30 минут или другими разрешенными дезинфицирующими средствами;
– твердые отходы следует инактивировать путем автоклавирования при температуре стерилизации не менее 132°С;
– не автоклавируйте растворы, содержащие азид натрия или гипохлорит натрия;
– утилизацию инактивированных отходов проводить согласно действующему национальному законодательству.

## 6. ХРАНЕНИЕ И ТРАНСПОРТИРОВАНИЕ

ИФА набор стабильный в течение срока годности, указанного на этикетке, если его хранить при температуре 2-8°С. Транспортировать набор при температуре 2-8°С. Допускается одноразовая транспортировка при температуре не выше 23°С в течение двух суток.

## 7. РЕКОМЕНДАЦИИ ПО ОТБОРУ, ТРАНСПОРТИРОВКЕ И ХРАНЕНИЮ ОБРАЗЦОВ

Кровь необходимо собирать из вены в стерильную пробирку. Пробирка должна быть промаркирована с указанием идентификационных данных пациента и дать забор образца. Цельную кровь до отделения сыворотки можно хранить до 24 часов при температуре 2-8°С, не допуская замораживания.

Сыворотку или плазму можно хранить при температуре 2-8°С в пределах 3 суток. Допускается более длительное хранение замороженной сыворотки при температуре -20°С или -70°С. Перед использованием замороженные образцы следует разморозить и выдержать при комнатной температуре в течение 30 минут. После размораживания образцы следует перемешать для достижения однородности. Избегать повторной заморозки-оттаивания исследуемых образцов. При помутнении сыворотки (или плазмы) освобождаются от нерастворимых включений центрифугированием при 3000 об/мин в течение 10-15 минут. Не следует использовать образцы сывороток с выраженной липидемией, гемолизом, а также бактериальным ростом.

Образцы сывороток транспортировать в термоизоляционных контейнерах. Для этого закрытые промаркированные пробирки необходимо поместить в полиэтиленовый пакет, закрыть плотно и положить в центре термоконтейнера. Замороженные хладагенты положить на дно вдоль боковых стен термоконтейнера и покрыть ими образцы сывороток.

## 8. ПОДГОТОВКА РЕАГЕНТОВ

*Примечание: Перед использованием выдержите все компоненты набора ИФА при комнатной температуре 18-25°С в течение 30 минут!*

### 8.1. Подготовка планшета ИФА

Для предотвращения конденсации воды в лунках открывайте STRIPS только после выдерживания 30 минут при комнатной температуре. Раскройте вакуумную упаковку, отделите необходимое количество лунок, а остальные сразу же тщательно упаковайте с влагопоглотителем и храните плотно закрытыми на замок zip-lock при температуре 2-8°С. Хранение таким образом упакованного планшета обеспечивает его стабильность в течение 6 месяцев.

### 8.2. Приготовление промывочного раствора

Для приготовления промывочного раствора разведите 1:20 (1+19) дистиллированной или деионизированной водой, затем перемешайте. Например, 5 мл концентрата + 95 мл воды, что достаточно для 8 лунок. При наличии кристаллов в концентрате промывочного раствора прогрейте флакон при температуре 37°С до полного растворения кристаллов (15–20 минут). Разбавленный раствор можно хранить при температуре 2-8°С не более 7 суток.

## 9. ПРОЦЕДУРА АНАЛИЗА

9.1. Подготовьте необходимое количество лунок для анализа (четыре лунки для контроля и необходимое количество для исследуемых образцов), вставьте их в рамку планшета ИФА. Лунки с контролями обязательно включайте в каждую постановку анализа.

9.2. Заполните схему внесения образцов.

9.3. Приготовьте раствор для промывания согласно пункту 8.2.

9.4. Внесите во все лунки планшета по 80 µl DIL SAMPLE.

9.5. Внесите в лунки по 40 µl контролей и исследуемых образцов:
CONTROL + – в лунку A1,
CONTROL - – в лунки B1, C1, D1,
в остальные лунки – исследуемые образцы.

При внесении происходит изменение цвета раствора с коричневого на синий. Осторожно пипетируйте смесь в лунках, не допуская пенообразования.

9.6. Заклейте стрипы клейкой пленкой и инкубируйте в течение 60 минут при температуре 37°C.

9.7. По окончании инкубации аккуратно снимите клейкую пленку и промойте лунки пять раз с использованием автоматического промывателя или 8-канальной пипетки следующим образом:
– удалите содержимое лунок в контейнер для жидких отходов;
– наполните лунки стрипов не менее чем по 300 µl раствором для промывки, оставьте не менее 30 секунд;
– аспирируйте раствор из лунок. Остаточный объем раствора после каждого этапа аспирации должно составлять не более 5 µl;
– повторите процедуру промывки еще четыре раза;
– после последней аспирации избавьтесь от лишней влаги, постукивая планшетом по фильтровальной бумаге.

9.8. Внесите в лунки по 100 µl SOLN CONJ. Стрипы накройте новой клейкой пленкой и инкубируйте в течение 30 минут при температуре 37°C.

9.9. По окончании инкубации аккуратно снимите клейкую пленку и промойте лунки пять раз, как описано в пункте 9.7.

9.10. Внесите в лунки по 100 µl SOLN TMB, не касаясь дна и стенок лунок планшета.

9.11. Инкубируйте стрипы в течение 30 минут в темном месте при комнатной температуре 18-25°C. Не используйте клейкую пленку на данном этапе.

9.12. Внесите в лунки стрипов по 100 µl SOLN STOP для остановки ферментативной реакции, соблюдая ту же последовательность, что и при внесении SOLN TMB. При внесении происходит изменение цвета раствора с голубого на желтый, в лунках с прозрачным раствором несколько меняется оттенок.

9.13. Измерьте на ридере ОП в каждой лунке при длине волны 450/620-695 nm в течение 5 минут после остановки реакции. До проведения

измерения убедитесь в чистоте наружной поверхности дна лунок и отсутствии пузырьков.

*Учет результатов анализа можно проводить в одноволновом режиме при длине волны 450 nm, в этом случае оставьте лунку для установки бланка (в такую лунку внесите только* SOLN TMB *и* SOLN STOP*).*

## 10. УЧЕТ РЕЗУЛЬТАТОВ И ИХ ИНТЕРПРЕТАЦИЯ

### 10.1. Учет результатов анализа

Рассчитайте среднее значение ОП негативного контроля ($\overline{Nc}$) и уровень граничного значения (Cut off - CO).

$$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3; \quad CO = \overline{Nc} + 0,25$$

### 10.2. Контроль достоверности результатов анализа

Данные теста считаются достоверными, если они отвечают следующим требованиям:

| | | |
|---|---|---|
| CONTROL + | ОП ≥ 1,5 | |
| CONTROL - | ОП ≤ 0,100 | |
| CONTROL - | $\overline{Nc} \times 0,5 \le Ncn \le \overline{Nc} \times 2,0$ | где Ncn – ОП каждого повтора Nc |

Если одно из значений ОП негативного контроля выходит за пределы указанного выше интервала, его отбрасывают и рассчитывают $\overline{Nc}$ по остальным значениям ОП негативного контроля. Если более одного значения ОП негативного контроля не соответствует указанным требованиям, то тест считается некорректным и требует повторного выполнения.

### 10.3. Интерпретация результатов

| | | |
|---|---|---|
| $OD_{sample} \ge CO$ | ПОЛОЖИТЕЛЬНЫЙ* | , где $OD_{sample}$ - ОП образца |
| $OD_{sample} < CO$ | ОТРИЦАТЕЛЬНЫЙ | |

* Первоначально положительные образцы должны быть исследованы повторно в двух лунках набора ИФА «EQUI anti-HCV». После повторного тестирования положительными считаются образцы, оптическая плотность которых хотя бы в одном из повторов превышает предельное значение. Согласно рекомендациям ВОЗ, для диагностики гепатита С образцы, интерпретированные как положительные, должны быть дополнительно проверены на наличие РНК вируса гепатита С. Положительный результат на анти-ВГС антитела при отсутствии РНК вируса не может свидетельствовать об активной гепатитной инфекции. Если при повторном тестировании оптическая плотность образца в обоих повторах ниже граничного значения, такой образец считать отрицательным.

Результаты для образцов, ОП которых равно граничному значению или находится в пределах ±10%, следует интерпретировать осторожно. Такие образцы должны быть исследованы повторно в двух лунках набора «EQUI

anti-HCV». Если при повторном тестировании $OD_{sample}$ снова находится в пределах ±10% от граничного значения следует провести отбор и анализ нового образца.

## 11. ХАРАКТЕРИСТИКИ ТЕСТА

### 11.1. Аналитические характеристики

**Прецизионность**

*Повторяемость результатов в рамках одной постановки анализа (Intra assay repeatability)*

Коэффициент вариации (CV) для двух сывороток с разным уровнем специфических антител оценивали в 32 повторах на одной серии ИФА-наборов.

| № сыворотки | $ОП_{ср}$ | CV, % |
|---|---|---|
| 973/300 | 1,422 | 6,9 |
| 704/500 | 1,845 | 5,0 |

*Воспроизводимость результатов между разными постановками анализа (Inter assay reproducibility)*

Коэффициент вариации (CV) для двух сывороток с разным уровнем специфических антител оценивали в течение 4 дней в 4 постановках анализа, по 8 повторов в каждом анализе.

| № сыворотки | $ОП_{ср}$ | CV, % |
|---|---|---|
| 973/300 | 1,500 | 8,6 |
| 704/500 | 1,912 | 8,5 |

**Аналитическая специфичность**

На результат анализа не влияет присутствие в образце билирубина в концентрации до 0,1 mg/ml (172,3 µmol/l), гемоглобина в концентрации до 5 mg/ml и триглицеридов в концентрации до 10 mg/ml (11,3 mmol/l).

### 11.2. Диагностические характеристики

Для определения клинической чувствительности и специфичности ИФА-наборов «EQUI anti-HCV» использовали 67 образцов сывороток от пациентов с диагнозом ВГС и 300 образцов сывороток клинически здоровых доноров. Кроме того, были использованы коммерческие панели охарактеризованных образцов производства SeraCare Life Sciences Inc., а также стандартная панель «Стандарт АТ(+/-)ВГС-МБА» производства ООО «Медбиоальянс». Клиническая чувствительность ИФА-наборов «EQUI anti-HCV» составила 100%, клиническая специфичность – 99,7%.

Исследование характеристик метода по сравнению с аналогичным коммерческим ИФА-набором проводилось на целевой группе беременных женщин (169 образцов). Для этой выборки относительная специфичность набора «EQUI anti-HCV» составляла 100%, процент совпадения - 98,2%.

Положительная прогностическая ценность (PPV) ИФА-набора «EQUI anti-HCV» составляет 99,1%, отрицательная прогностическая ценность (NPV) – 100%.

## 12. ОГРАНИЧЕНИЕ АНАЛИЗА

Интерпретация результатов должна проводиться с учетом клинических проявлений и данных комплекса лабораторных исследований. Для диагностики острого, хронического или перенесенного гепатита С, оценки эффективности терапии рекомендуется дополнительно провести исследование образца на наличие РНК ВГС, антител к отдельным белкам ВГС и анти-ВГС IgM антител (например, в ИФА-наборах «EQUI anti-HCV Different» и «EQUI HCV IgM», соответственно), и оценить биохимические показатели сыворотки крови.

Современные методы выявления антител к ВГС не могут обеспечить выявления всех инфицированных пациентов. Негативный результат не исключает инфицирование вирусом гепатита С пациента, особенно если он проходит иммуносупрессивное лечение или инфицированный ВИЧ, а также на ранних стадиях гепатитной инфекции.

Любой из методов ИФА может допустить ложноположительную реакцию. Для исключения ложноположительных результатов рекомендуется провести верификационное исследование с определением РНК вируса гепатита С или антител к отдельным ВГС белкам.

## 13. ТРУДНОСТИ, КОТОРЫЕ МОГУТ ВОЗНИКНУТЬ ПРИ ПРОВЕДЕНИИ ИФА

***Высокий фон в лунках всего планшета может возникнуть из-за:***

– загрязненного промывателя;
– низкого качества или загрязнения воды;
– использования плохо помытой посуды;
– использования дезинфицирующих средств, содержащих хлор;
– использования загрязненных наконечников;
– увеличения времени инкубации или изменения температурного режима.

***Высокий фон в отдельных рядах может быть связан с:***

– повторным внесением раствора ТМБ;
– загрязнением конуса автоматической пипетки раствором конъюгата;
– загрязнением одного из каналов промывателя.

***Полученное значение ОП положительного контроля ниже установленной границы, если***:

– неправильно приготовлен или не внесен один из реагентов (раствор конъюгата или раствор ТМБ);
– сокращено время инкубации на одном из этапов.

***Интенсивность окрашивания лунок не соответствует полученной оптической плотности.*** Это может свидетельствовать о смещенном оптическом луче.

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 Производитель

IVD Медицинское изделие для диагностики *in vitro*

REF Номер по каталогу

 Дата изготовления

 Использовать до

LOT Код партии

 Температурное ограничение

 Содержит достаточно для (n-) испытаний

 Предостережение, ознакомиться с сопроводительными документами

 Ознакомление с инструкцией по применению

 Беречь от прямых солнечных лучей

 Знак соответствия техническим регламентам

Редакция 7 от 11.10.2021г.

*С вопросами и пожеланиями по работе набора обращайтесь к производителю:*

ООО «Эквитестлаб»
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e-mail: info@equitest.com.ua, www.equitest.com.ua

## СХЕМА ПРОВЕДЕНИЯ АНАЛИЗА

| |
|---|
| Выдержать реагенты 30 min при температуре 18-25 °C |
| В лунки планшета внести по 80 µl DIL SAMPLE (коричневый цвет) |
| Внести по 40 µl контролей и исследуемых образцов в лунки: A1 – CONTROL +, B1, C1, D1 – CONTROL –, E1 и в остальные лунки - исследуемые образцы (происходит изменение цвета с коричневого на синий) |
| Заклеить стрипы пленкой, инкубировать **60 min при температуре 37°C** |
| Промыть лунки 5 раз приготовленным 1:20 (1+19) раствором для промывания TRITON (300 µl в лунку) |
| В лунки стрипов внести по 100 µl SOLN CONJ (зеленый цвет) |
| Заклеить стрипы пленкой, инкубировать **30 min при температуре 37°C** |
| Промыть лунки 5 раз приготовленным 1:20 (1+19) раствором для промывания TRITON (300 µl в лунку) |
| В лунки стрипов внести по 100 µl SOLN TMB |
| Инкубировать в течение **30 min в темноте при температуре 18-25°C** |
| В лунки стрипов внести по 100 µl SOLN STOP (происходит изменение цвета с голубого на желтый) |
| Измерить оптическую плотность (ОП) на спектрофотометре при 450/620-695 nm |

## УЧЕТ РЕЗУЛЬТАТОВ АНАЛИЗА

$\overline{Nc}$ = (Nc1 + Nc2 + Nc3)/3;

CO = Nc + 0,25;

$\overline{Nc}$ - Среднее значение ОП 3-х CONTROL –

CO - Уровень граничного значения (Cut off)

## ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

| | |
|---|---|
| $OD_{sample} \geq CO$ | ПОЛОЖИТЕЛЬНЫЙ |
| $OD_{sample} < CO$ | ОТРИЦАТЕЛЬНЫЙ |

**EKVITESTLAB LLC**

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**e-mail: info@equitest.com.ua**
**www.equitest.com.ua**

# STATEMENT

We, EKVITESTLAB LLC, having a registered office at Velyka Vasylkivska street 114, Kyiv, 03150, Ukraine assign SRL SANMEDICO having a registered office at A. Corobceanu street 7A, apt. 9, Chişinău MD-2012, Moldova, as authorized representative in correspondence with the conditions of directive 98/79/EEC.

We declare that the company mentioned above is authorized to register, notify, renew or modify the registration of medical devices on the territory of the Republic of Moldova.

Date: 03 January 2023

Signature: _____

Director, Anna Yurchuk

# Declaration of Conformity

According to annex III of the Council Directive 98/79/EC on in vitro diagnostic medical device We,


**EKVITESTLAB LLC**

**Velyka Vasylkivska St. 114, Kyiv, Ukraine, 03150, tel. 0(800)31-89-87; +38 (044)334-89-87**

**e-mail: info@equitest.com.ua, web-site: www.equitest.com.ua**


Declare under our sole responsibility that the following in vitro diagnostic medical devices other than those covered by annex II and devices for performance evaluation

**EQUI HAV IgM - ELISA kit for the qualitative detection of IgM antibodies to hepatitis A virus, REF EI-031**

Meet the provisions of the Council Directive 98/79/EC concerning medical devices which apply to them.

Undersigned declares to fulfill the obligations imposed by Annex III section 2 to 5:

- availability of the technical documentation set in Annex III (section 3), allowing the assessment of conformity of the product with the requirements of the Directive.
- the manufacturer shall take necessary measures to ensure that the manufacturing process follows the principles of quality assurance as appropriate for the products manufactured (Annex III section 4).
- the manufacturer shall institute and keep up to date a systematic procedure to review experience gained from devices in the post-production phase and to implement appropriate means to apply any necessary corrective actions (Annex III section 5).

Conformity assessment was performed according to Article 9 (7) and Annex III, section 3.

Our current Quality System is formatted to international standards:

- **ISO 13485:2016 «Medical devices — Quality management systems — Requirements for regulatory purposes»**

Corporate Contact Information

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Stamp


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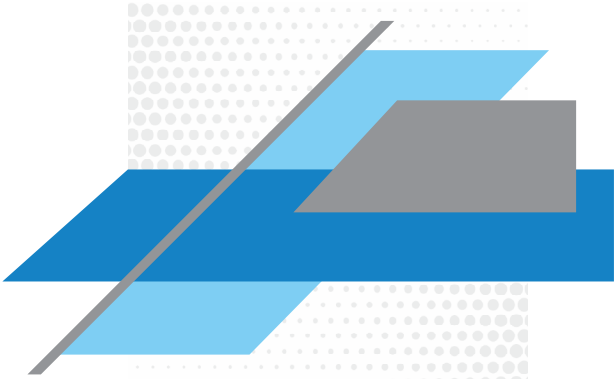
Representative: Mr. Gideon ELKAYAM (CEO)

# HAV IgM

**ELISA kit for the qualitative detection of IgM antibodies to hepatitis A virus**

Instruction for use



IVD

REF EI-031

Σ 96 tests

CE

# EQUI HAV IgM

ELISA kit for the qualitative detection
of IgM antibodies to hepatitis A virus

## 1. INTENDED USE

The «EQUI HAV IgM» is ELISA kit intended for the qualitative detection of IgM antibodies to hepatitis A virus in human serum or plasma by enzyme-linked immunosorbent assay (ELISA) to diagnose acute hepatitis A. The testing procedure is designed for both manual arrangement with automatic pipettes and standard equipment, and for automated «open» immunoassay analysers.

**Target group:** blood or organ donors; pregnant women and children born to infected mothers; patients with symptoms of liver disease.

**Usage:** ELISA kit is used in clinical diagnostic laboratories, blood transfusion stations, as well as in other institutions working in the field of *in vitro* diagnostics.

## 2. CLINICAL SIGNIFICANCE

One of the most common foodborne infections is hepatitis A. The hepatitis A virus (HAV) causes acute liver disease, which can be mild or severe. Unlike hepatitis B and C, this hepatitis does not become chronic, but can lead to acute liver failure, which is characterized by high mortality.

Hepatitis A virus is a small shellless RNA virus from the *Picornaviridae* family. It is characterized by being highly stable in different environments and can be stored at + 4 ° C for several months, but becomes inactive after 5 minutes at 100 ° C. The virus replicates in liver cells, and then is released through the bile into the environment with the fecal masses of the infected person. The cellular immune response to HAV infection leads to the destruction of hepatocytes, liver dysfunction and the development of symptoms, typical for other types of hepatitis.

Acute hepatitis A, even with the clinical manifestations, does not differ from other viral hepatitis. Therefore, serological markers of infection are used for diagnosis, namely the detection of specific antibodies to HAV antigens. IgM antibodies are detected in the serum 1-2 weeks after infection, with the onset of symptoms or a few days before. In the maximum titer of anti-CAA IgM are detected in the jaundice period, after that their level gradually decreases. In most patients, specific IgM ceases to be detected after 6 months, and may occasionally circulate in the blood for more than a year. IgG antibodies to HAV antigens begin to be released shortly after IgM antibodies and stay in the blood throughout life. Also, specific IgG is produced after a vaccination. Detection of IgG antibodies to CAA indicates the formation of a stable immunity due to infection or immunization.

## 3. ANALYSIS PRINCIPLE

Detection of specific IgM antibodies to hepatitis A virus in the «EQUI HAV IgM» ELISA kit is based on the principle of «IgM capture» of solid-phase ELISA in a two-stage incubation. Monoclonal antibodies specific for human IgM immunoglobulins are adsorbed into the wells of the plate. During the first step of incubation of the

test samples in the wells of the ELISA plate, IgM immunoglobulins, if present in the samples, bind to monoclonal antibodies in the solid phase. The wells are washed to remove unbound components, leaving only specific antibody-antibody complexes. A mixture of HAV antigen and peroxidase conjugate of HAV-specific antibodies that bind to solid-phase immune complexes is then added. Unbound components are removed during washing. Immune complexes are detected by adding a solution of chromogen 3,3', 5,5'-tetramethylbenzidine (TMB) with hydrogen peroxide. After a 30-minute incubation, the reaction is halted by adding a stop solution. Optical density (OG) in the wells is determined on a spectrophotometer at a wavelength of 450 / 620-695 nm. The intensity of the yellow color is proportional to the number of antibodies in the sample.

## 4. MATERIALS AND EQUIPMENT

### 4.1. Contents of the ELISA kit

| | | |
|---|---|---|
| STRIPS | 1 x 96 wells | **Microplate**<br>Each plate well is coated with monoclonal antibodies specific for human IgM immunoglobulins. The wells are detachable. After the first opening, store unused strips in the package at 2-8 °C for a maximum of 6 months |
| CONTROL + | 1 x 0,25 ml | **Positive control**<br>The solution of human IgM immunoglobulins crosslinked with monoclonal antibodies specific for horseradish peroxidase, with a preservative (pink). Store at 2-8 °C |
| CONTROL - | 1 x 0,6 ml | **Negative control**<br>Negative human serum with a preservative (yellow). Store at 2-8 °C |
| DIL SAMPLE | 1 x 13 ml | **Serum dilution solution**<br>Buffer solution with monoclonal antibodies to human IgG, milk extract, detergent and preservative (brown). Store at 2-8 °C |
| CONJ 11x | 1 x 1,3 ml | **Conjugate (11x concentrated)**<br>11-fold concentrate of conjugate of antibodies to hepatitis A virus with horseradish peroxidase in buffer solution with stabilizers (purple).<br>Dilute the conjugate (11x) 1:11 with the conjugate dilution solution before use (eg 100 µl concentrate + 1 ml conjugate dilution solution, enough for 8 wells). Diluted solution should be stored at 2-8 ° C for no more than 1 day |
| DIL CONJ | 1 x 13 ml | **Conjugate dilution solution**<br>Buffer solution of inactivated hepatitis A virus antigen with detergent and preservative (yellow). Store at 2-8 °C |
| SOLN TMB | 1 x 13 ml | **TMB solution (ready to use)**<br>TMB solution, $H_2O_2$, a stabilizer, a preservative (colourless). Store at 2-8 °C |

| | | |
|---|---|---|
| TWEEN WASH 20x | 1 x 50 ml | **Washing solution TWEEN (20x concentrated)** 20-fold phosphate buffer concentrate with Tween-20 (colourless). Dilute TWEEN detergent (20x) at 1:20 with distilled or deionized water (e. g., 5 mL of concentrate + 95 mL of water for 8 wells) before use. Store the diluted solution at 2-8 °C for a maximum of 7 days |
| SOLN STOP | 1 x 13 ml | **Stop Solution (ready to use)** 0.5 mol $H_2SO_4$ solution (colourless). Store at 2-8 °C |

The ELISA kit also includes adhesive films (2 items), sample application plan (1 item), checklist, and instruction for use.

### 4.2. Optional reagents, materials and equipment

Automatic single and multichannel pipettes 10–1000 µL, tips, volumetric laboratory glassware (10–1,000 mL), deionized or distilled water, thermostat at 37 °C, automatic or semi-automatic plate washer, spectrophotometer (reader) for microplates at 450/620-695 nm, appropriate containers for potentially contaminated waste, timer, filter paper, disposable powder-free gloves, disinfectants.

## 5. PRECAUTIONS AND SAFETY

### 5.1. Precautions

*Be sure to read the instructions for use carefully before the test. The validity of the test results depends on strict following of the test procedure.*

– do not use the ELISA kit components after the expiry date;
– do not use for analysis or mix components of different batches, components of kits for different nosologies, or reagents from other manufacturers with the «EQUI HAV IgM» ELISA kit;
– do not freeze the ELISA kit or its contents;
– after using a reagent, close each vial with its cap;
– when washing, control filling and complete aspiration of solution from the wells;
– use a new pipette tip each time you add samples or reagents;
– prevent direct sunlight from reaching the reagents from the ELISA kit;
– SOLN TMB solution must be colourless before use. Do not use the solution if its colour is blue or yellow. Avoid contact of SOLN TMB with metals or metal ions. Use only clean glassware thoroughly rinsed with distilled water;
– do not use reagents with colour not in line with para. 4.1;
– under no circumstances should the same glassware be used for conjugate solution and SOLN TMB;
– do not evaluate the test results visually (without a reader);
– any optional equipment that is in direct contact with biological material or kit components should be considered contaminated and requires cleaning and decontamination;
– the ELISA kit includes materials for 96 tests. Dispose of the used components as well as any remaining unused components.

**5.2. Safety requirements**

– all reagents in the ELISA kit are for laboratory professional use for *in vitro* diagnosis only and may only be used by qualified personnel;
– conduct the tests in disposable powder-free gloves and goggles only;
– do not eat, drink, smoke, or apply make-up in the test room;
– do not mouth-pipette the solutions;
– controls from the «EQUI HAV IgM» ELISA kit have been tested and found to be for anti-HIV1/2, anti-HCV and anti-*Treponema pallidum* antibodies and HBsAg negative; however, controls and test samples should be handled as potentially hazardous infectious materials;
– some of the kit components contain low concentrations of harmful substances and can damage skin or mucoga. In case of contact of SOLN TMB, SOLN STOP and conjugate solution with mucous membranes or skin, immediately wash the affected area with plenty of water;
– in case of spillage of acid-free solutions, e. g. sera, treat the surface with a disinfectant solution and then wipe dry with filter paper. Otherwise first neutralize acid with sodium bicarbonate solution and then wipe the surface dry as described above.

**5.3. Waste inactivation and disposal**

– the liquid waste must be inactivated, for example, with hydrogen peroxide solution at the final concentration of 6% for 3 hours at room temperature, or with sodium hypochlorite at the final concentration of 5% for 30 minutes, or with other approved disinfectants;
– the solid waste must be inactivated by autoclaving at a temperature not less than 132°C;
– do not autoclave the solutions that contain sodium azide or sodium hypochlorite;
– disposal of inactivated waste must be conducted due to national laws and regulations.

## 6. STORAGE AND STABILITY

ELISA kit is stable up to the expiry date stated on the label when stored at 2-8°C. The kit should be transported at 2-8°C. Single transportation at a temperature up to 23°C for two days is possible.

## 7. SAMPLE COLLECTION, TRANSPORTATION AND STORAGE GUIDELINES

Collect blood from the vein into the sterile test tube. Test tube must be marked with patient ID and date of sample collecting. Blood before serum separation can be stored at 2-8 ºC for 24 hours, avoiding freezing.

Serum or plasma can be stored at 2-8 °C for maximum 3 days. Frozen serum can be stored for longer periods of time at -20 °C or -70 °C. Thaw frozen samples and keep them at room temperature for 30 minutes before use. After thawing, the stir samples to achieve homogeneity. Avoid repeated freezing-thawing cycles for test samples. If serum (or plasma) is turbid, remove insoluble inclusions by

centrifugation at 3000 rpm for 10-15 minutes. Do not use serum samples with hyperlipidemia, hemolysis, and bacterial growth.

Transport serum samples in insulated containers. To do that, put closed labelled tubes in a plastic bag, tightly seal it and place in the centre of an insulated container.Put the frozen cold packs on the bottom, along the side walls of the insulated container and on top of the serum samples.

## 8. REAGENT PREPARATION

*NOTE! It is very important to keep all ELISA kit components for at least 30 min at room temperature 18-25 ºC before the assay!*

### 8.1. Microplate preparation

To prevent water condensation in the wells, keep the STRIPS for 30 minutes at a room temperature before opening. Open the vacuum pack, detach the appropriate number of wells, and carefully pack the remaining wells with a desiccant and store tightly zip-locked at 2-8 °C. Storing the packed plate this way ensures its stability for 6 months.

### 8.2. Washing solution preparation

To prepare detergent, dilute TWEEN WASH 20x at 1:20 (1+19) with distilled or deionized water and stir. E. g., 5 mL of concentrate + 95 mL of water, which is enough for 8 wells. If there are crystals present in the detergent concentrate, heat the vial at 37 °C until the crystals dissolve completely (15–20 minutes). Store the diluted solution at 2-8 °C for a maximum of 7 days.

### 8.3. Conjugate solution preparation

Working dilution of the conjugate is prepared as follows: dilute CONJ 11x (purple) in a clean vial of solution DIL CONJ (yelow) in the ratio 1:11 (ie, 1 + 10), the solution turns green. For example, for 8 well analysis add to 1 ml DIL CONJ 100 µl CONJ 11x. The solution of the conjugate in the working dilution is stable during the day when stored at 2-8 °C.

## 9. ASSAY PROCEDURE

9.1. Prepare the necessary number of wells (four wells for controls and a necessary number of wells for test samples) and insert them into the ELISA plate frame. Be sure to add control wells in every test run.

9.2. Fill in the sample application plan.

9.3. Prepare the detergent as per para. 8.2.

9.4. Add 90 µL of DIL SAMPLE into each plate well.

9.5. Add 10 µL of controls and test samples into the wells:
CONTROL + – into well A1,
CONTROL - – into wells B1, C1 and D1,
and test samples into the remaining wells.
At the time of adding, the solution changes its colour from brown to blue. Pipette the mix in the wells carefully to avoid foaming.

9.6. Cover the strips up with adhesive film and incubate for **30 minutes at 37 °C**.

9.7. Remove and discard the adhesive film and wash all wells 5 times with automatic washer or 8-channel pipette as follows:
– aspirate the content of all wells into a liquid waste container;
– add a minimum of 300 µl of diluted washing solution to each well, soak each well for30 seconds;
– aspirate the content of all wells again. The residual volume after every aspiration should be less than 5 µl;
– repeat the washing step 4 more times;
– after the final aspiration, eliminate extra moisture by tapping the plate against a piece of filter paper.

9.8. Prepare conjugate solution as per para. 8.3.

9.9. Add 100 µL of conjugate solution into each well. Cover the strips with a new piece of adhesive film and incubate for **60 minutes at 37 °C**.

9.10. Following incubation, remove the film carefully and wash the wells five times as described in para. 9.7.

9.11. Add 100 µL of SOLN TMB into the wells; do not touch the bottom and the walls of the plate wells.

9.12. Incubate the strips for **30 minutes** in a dark place at a room temperature of 18-25 °C. Do not use adhesive film at this stage.

9.13. Add 100 µL of SOLN STOP into each strip well to stop the enzymatic reaction; adhere to the same sequence of actions as when adding SOLN TMB. At the time of adding,the solution colour changes from blue to yellow, and clear solution slightly changes its shade.

9.14. Measure the optical density (OD) of the wells at 450/620-695 nm wavelength using an ELISA microplate reader within 5 minutes after stopping the reaction. Pay attention to the cleanness of the plate bottom and the absence of bubbles in the wells before reading.

*Measurement at the single wavelength of 450 nm is possible, in that case, it is needed to leave one well for blank (only SOLN TMB and SOLN STOP must be added in blank well).*

## 10. CALCULATION AND INTERPRETATION OF RESULTS

### 10.1. Calculation of results

Calculate the average OD of the negative control ($\overline{Nc}$), Cut off (CO) and a sample positivity index ($IP_{sample}$).

$$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3; \quad CO = \overline{Nc} + 0,3$$

$$IP_{sample} = OD_{sample}/CO, \text{ where } OD_{sample} \text{ is the OD sample.}$$

### 10.2. Quality control (assay validation)

The test results are considered valid if they meet the following requirements:

| | | |
|---|---|---|
| CONTROL + | OD ≥ 1,2 | |
| CONTROL - | OD ≤ 0,150 | |
| CONTROL - | $\overline{Nc} \times 0,5 \leq Ncn \leq \overline{Nc} \times 2,0$ | where Ncn is the OD for each Nc run |

If any of the OD values for the negative control is beyond the above interval, it should be discarded, and $\overline{Nc}$ is calculated based on the remaining OD values for the negative control. If several OD values for the negative control fail to meet the above requirements, the test is considered invalid and requires a new run.

### 10.3. Interpretation of results

| | |
|---|---|
| $IP_{sample} > 1,1$ | POSITIVE |
| $0,9 \leq IP_{sample} \leq 1,1$ | BORDERLINE* |
| $IP_{sample} < 0,9$ | NEGATIVE |

* Uncertain samples are recommended to be re-examined in two wells of the ELISA kit. If the results are again uncertain, a new sample should be selected and analyzed in 2-4 weeks. In case of repeated indeterminate results, such samples shall be considered negative.

## 11. PERFORMANCE CHARACTERISTICS

### 11.1. Analytical performance characteristics

**Precision of measurement**

*Intra assay repeatability*

The coefficient of variation (CV) for two sera with different levels of specific antibodies was evaluated in 32 replicates on one series of ELISA kits.

| Sample No. | $OD_{av}$ | $IP_{av}$ | CV, % |
|---|---|---|---|
| 37s/2 | 1,576 | 4,85 | 5,3 |
| 24s | 2,462 | 7,57 | 4,3 |

*Inter assay reproducibility*

The coefficient of variation (CV) for two sera with different levels of specific antibodies was evaluated for 4 days in 4 sets of analysis, 8 replicates in each analysis.

| Sample No. | $OD_{av}$ | $IP_{av}$ | CV, % |
|---|---|---|---|
| 37s/2 | 1,600 | 4,78 | 6,3 |
| 24s | 2,463 | 7,36 | 7,1 |

**Analytical specificity**

The test results are not affected by bilirubin at up to 0.21 mg/mL (361.8 µmol/L), haemoglobin at up to 10 mg/mL and triglycerides at up to 10 mg/mL (11.3 mmol/l) present in the sample.

### 11.2. Diagnostic characteristics

The diagnostic characteristics of the ELISA kit were evaluated by examining a set of 30 samples containing IgM antibodies to hepatitis A virus, a set of donor serum samples (188 samples) and samples of PHT202 Anti-Hepatitis A Virus (HAVed) panel. Performance Panel (contains 8 positive and 13 negative samples) - a total of 239 samples - was compared to similar commercial kits. For this set (239 samples) the relative sensitivity of the «EQUI HAV IgM» ELISA kit is 100%, the relative specificity - 100%, the percentage of coincidence - 100%.

## 12. LIMITATIONS OF ASSAY

A positive result in the «EQUI HAV IgM» ELISA kit is the evidence that the patient has IgM antibodies specific for hepatitis A virus. Anti-CAA specific IgM antibodies are usually markers of active replication of hepatitis A virus.

In order to counteract the false-positive results caused by the presence of autoantibodies specific for class G immunoglobulins (rheumatoid factor) in human serum samples, the kit uses a special block component that prevents the formation of immune complexes with anti-human antibodies in the solid phase.

The final diagnosis cannot be established solely on the basis of serological test results. When making a diagnosis the results of a set of laboratory and instrumental studies, as well as clinical manifestations of the disease should all be taken into account.

## 13. DIFFICULTIES THAT CAN OCCUR DURING THE ASSAY PROCEDURE

| Possible reasons | Solution |
|---|---|
| ***High background in all wells*** | |
| Contaminated washer | Clean the washer head and rinse according to the instructions for use |
| Poor quality or contaminated water | Use purified water with specific resistance ≥ 10 MΩ · cm |
| Use of poorly washed glassware | Use chemically clean utensils |
| Use of chlorinated disinfectants | Do not use chlorine disinfectants |
| Use of contaminated tips | Use new tips |
| Increased incubation times or change in the temperature conditions | Adhere to the incubation regime according to the instructions for use |
| ***High background in a row of wells*** | |
| Repeat application of TMB solution | TMB solution should be applied once |
| Contamination of the automatic pipette nozzle with conjugate solution | Clean the pipette and dial carefully liquid |
| Contamination of one of the washer's channel | Clean the flush channel, rinse washer |

| | |
|---|---|
| ***Received OD of the positive control is below the border value*** | |
| One of the reagents (conjugate solution or TMB solution) was not prepared in a correct way or was not added | Re-conduct ELISA, pay attention to the correctness of the introduction of these reagents |
| Reduced incubation times at any stage | Incubate according to instructions for use |
| ***The colour density of the wells fails to meet the obtained optical density value*** | |
| This may suggest that the optical beam has been displaced | Check the correct operation of the reader |

## 14. TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

In case of technical problems, you can obtain assistance by contacting the manufacturer.

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| | |
|---|---|
|  | Manufacturer |
|  | Authorized Representative in the European Community |
|  | In vitro diagnostic medical device |
|  | Catalogue number |
|  | Date of manufacture |
|  | Use by date |
|  | Batch code |
|  | Temperature limit |
|  | Contains sufficient for <n> tests |
|  | Caution |
|  | Non-Sterile |
|  | Consult instructions for use |
|  | Keep away from sunlight |
|  | Keep dry |
| CE | Compliance with EU safety requirements |

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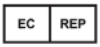
For questions and suggestions regarding the ELISA kit contact:

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## ASSAY PROCEDURE SCHEME

| |
|---|
| Keep all reagents for 30 min at temperature18-25°C before use |
| Dispense 90 µl DIL SAMPLE into the wells (brown) |
| Add to 10 µl of controls and samples into the wells: A1 – CONTROL +, B1, C1, D1 – CONTROL –, other wells – examined samples (change of colour from brown to blue) |
| Cover strips with an adhesive film, incubate for **30 min at 37°C** |
| Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well) |
| Add 100 µl of prepared 1:11 (1+10) conjugate solution into all wells (green) |
| Cover strips with an adhesive film, incubate for **60 min at 37°C** |
| Rinse the wells 5 times with prepared 1:20 (1+19) washing solution TWEEN (300 µl per well) |
| Add 100 µl of SOLN TMB into all wells |
| Incubate for **30 min in the dark at 18-25°C** |
| Add 100 µl of SOLN STOP into all wells (change of colour from blue to yellow) |
| Measure the optical density (OD) with an ELISA microplate reader at 450/620-695 nm |

## CALCULATION OF RESULTS

$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3;$

$CO = \overline{Nc} + 0,3;$

$IP_{sample} = OD_{sample}/CO$

$\overline{Nc}$ - the average value of OD 3-x CONTROL –

CO - Cut off

$IP_{sample}$ - sample positivity index

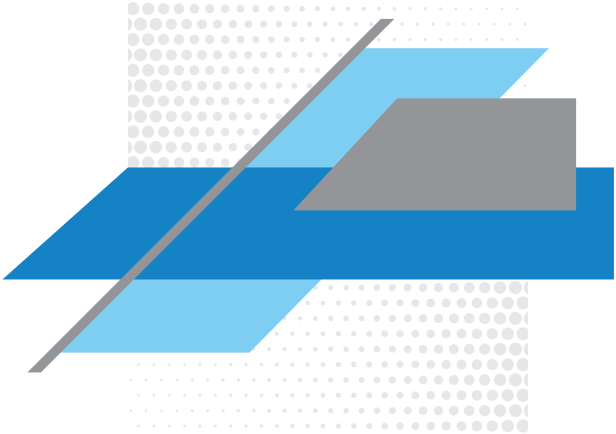
## INTERPRETATION OF RESULTS

| | |
|---|---|
| $IP_{sample} > 1,1$ | POSITIVE |
| $0,9 \leq IP_{sample} \leq 1,1$ | BORDERLINE |
| $IP_{sample} < 0,9$ | NEGATIVE |

# HBsAg

**ИФА-набор для качественного обнаружения поверхностного антигена вируса гепатита B**

Инструкция по применению



IVD

REF
EI-011

96
анализов

UA.TR.061

# EQUI HBsAg

ИФА-набор для качественного обнаружения поверхностного антигена вируса гепатита В

## 1. НАЗНАЧЕНИЕ

Набор «EQUI HBsAg» предназначен для качественного обнаружения поверхностного антигена вируса гепатита В (HBsAg) в сыворотке или плазме крови человека методом иммуноферментного анализа (ИФА) с целью диагностики гепатита В и скрининга донорской крови. Процедура анализа рассчитана как для ручной постановки с автоматическими пипетками и стандартным оборудованием, так и для автоматического иммуноферментного анализатора «открытого» типа.

**Целевая группа:** доноры; лица-потребители инъекционных наркотиков; реципиенты крови или органов; беременные женщины; дети, рожденные от инфицированных матерей; лица, инфицированные ВИЧ; пациенты с симптомами заболеваний печени; пациенты гемодиализа.

**Применение:** ИФА-набор применяется в клинических диагностических лабораториях, станциях переливания крови, а также в других учреждениях, работающих в области *in vitro* диагностики.

## 2. КЛИНИЧЕСКОЕ ЗНАЧЕНИЕ

Одним из распространенных заболеваний печени является гепатит В. Его этиологический агент – вирус гепатита В (ВГВ). ВГВ относится к семейству Hepadnaviridae и содержит двухцепочечную ДНК. Инфекционной формой вируса являются так называемые частицы Дейна диаметром 42-49 nm, в белковом составе которых основными являются поверхностный антиген (HBsAg) и коровой антиген (HBcAg).

Клиническая картина гепатита В не позволяет диагностировать его длительное время и отличить от других вирусных гепатитов. Поэтому для скрининговых исследований и подтверждения диагноза важную роль играет лабораторная диагностика, особенно выявление антигенов ВГВ и антител к ним методом ИФА. Первым и основным маркером гепатита В является HBsAg, проявляющийся в крови через 3-5 недель после инфицирования. Приблизительно в то же время в крови можно обнаружить ДНК ВГВ и HBeAg, который считается маркером активной репликации вируса и «заразности» крови. ВОЗ рекомендует проводить проверку всей донорской крови на HBsAg, чтобы предотвратить трансмиссивную передачу ВГВ. Через 2-3 недели после появления HBsAg появляются антитела IgM к коровому антигену HBcAg, а вскоре после них – анти-HBcore IgG, быстро достигающие высоких уровней. Выздоровление от острого гепатита В сопровождается выведением вируса из организма, перестают выявляться HBsAg и анти-HBc IgM, появляются антитела к HBeAg. Антитела IgG к коровому антигену персистируют в течение всей жизни и являются маркером имеющегося или перенесенного гепатита В, их уровень в крови снижается медленно. Через несколько месяцев после

исчезновения из крови HBsAg начинают выявляться анти-HBs антитела, свидетельствующие о перенесенном гепатите В и наличии иммунитета. В период «серологического окна» между выводом HBsAg и появлением анти-HBs антител маркером инфекции ВГВ являются суммарные антитела к коровому антигену, также могут проявляться анти-HBe антитела.

Если после острой фазы не происходит элиминация вируса и не появляются анти-HBs антитела, развивается хронический гепатит В. HBsAg продолжает определяться более 6 месяцев, его количество в крови может значительно колебаться. На репликативной стадии хронического гепатита В находится ДНК вируса и HBeAg, антител к HBeAg нет.

ВОЗ рекомендует диагностировать острый гепатит В при наличии HBsAg и антител IgM к HBcAg, а хронический – при устойчивом присутствии HBsAg в течение не менее шести месяцев.

Главным средством профилактики гепатита В является вакцинация, рекомендованная в первую очередь новорожденным. После вакцинации организмом продуцируются анти-HBs антитела и формируется иммунитет у лиц, не соприкасавшихся с вирусом гепатита В. Наличие анти-HBs антител на уровне более 10 IU/l (МЕ/л) принято считать нижним пределом протективного иммунитета вследствие вакцинации или перенесенного гепатита В.

## 3. ПРИНЦИП АНАЛИЗА

Обнаружение HBsAg в ИФА-наборе «EQUI HBsAg» базируется на принципе «сэндвич»-варианта твердофазного ИФА в одноэтапной инкубации. В лунках планшета засорбированы моноклональные антитела, специфические к HBsAg. В каждую лунку добавляются образцы сыворотки или плазмы пациента и конъюгат специфических к HBsAg антител с пероксидазой хрена. Во время инкубации исследуемых образцов и пероксидазного конъюгата в лунках планшета HBsAg, при наличии в образцах, связывается как с первыми антителами на твердой фазе, так и со вторыми антителами, конъюгированными с пероксидазой хрена, образуя «сэндвич» антитело -антиген-антитело. Несвязанные компоненты удаляются при отмывании. Иммунные комплексы обнаруживаются путем добавления раствора хромогена 3,3',5,5'-тетраметилбензидина (ТМБ) с перекисью водорода. После 30-минутной инкубации реакция останавливается добавлением стоп-раствора. Оптическая плотность (ОП) в лунках определяется на спектрофотометре при длине волны 450/620-695 nm. Интенсивность желтой окраски пропорциональна количеству HBsAg в образце.

## 4. МАТЕРИАЛЫ И ОБОРУДОВАНИЕ

### 4.1. Состав набора

| | | |
|---|---|---|
| STRIPS | 1 x 96 лунок | **Планшет ИФА**<br>В каждой лунке планшета засорбированы моноклональные антитела к HBsAg. Лунки можно отделять. После первого открытия храните неиспользованные стрипы в упаковке при температуре 2-8°C не более 6 месяцев |
| CONTROL + | 1 x 1,6 ml | **Позитивный контроль**<br>Раствор поверхностного антигена вируса гепатита B в буфере с альбумином и консервантом (розовый). Хранить при температуре 2-8°C |
| CONTROL - | 2 x 1,6 ml | **Негативный контроль**<br>Негативная сыворотка крови человека с консервантом (желтый). Хранить при температуре 2-8°C |
| CONJ 11x | 1 x 0,8 ml | **Конъюгат (11x концентрат)**<br>11-кратный концентрат конъюгата моноклональных антител к HBsAg с пероксидазой хрена в буферном растворе со стабилизаторами и консервантом (фиолетовый). Развести конъюгат (11x) 1:11 раствором для разведения конъюгата перед использованием (например, 50 µl концентрата + 500 µl раствора для разведения конъюгата, достаточно для 8 лунок). Разбавленный раствор хранить при температуре 2-8°C не более 1 суток |
| DIL CONJ | 1 x 8 ml | **Раствор для разведения конъюгата**<br>Буферный раствор с белками сыворотки крови крупного рогатого скота и иммуноглобулинами мыши с консервантом (розовый). Хранить при температуре 2-8°C |
| SOLN TMB | 1 x 13 ml | **Раствор ТМБ (готов к использованию)**<br>Раствор ТМБ, $H_2O_2$, стабилизатор, консервант (бесцветный). Хранить при температуре 2-8°C |
| TWEEN WASH 20x | 1 x 50 ml | **Раствор для промывки TWEEN (20x концентрат)**<br>20-кратный концентрат фосфатного буфера с Твином-20 (бесцветный). Развести раствор для промывки TWEEN (20x) 1:20 дистиллированной или деионизированной водой (например, 5 ml концентрата + 95 ml воды для 8 лунок) перед использованием. Разбавленный раствор хранить при температуре 2-8°C не более 7 суток |
| SOLN STOP | 1 x 13 ml | **Стоп-раствор (готов к использованию)**<br>Раствор 0,5 mol $H_2SO_4$ (бесцветный). Хранить при температуре 2-8°C |

В состав набора входят: клейкая пленка (1 шт.), схема внесения образцов (1 шт.), лист контрольных испытаний и инструкция по применению.

### 4.2.Дополнительные реактивы, материалы и оборудование

Автоматические пипетки переменного объема на 10–1000 µl и наконечники к ним, мерная лабораторная посуда (10–1000 ml), деионизированная или дистиллированная вода, термошейкер на 37°C или термостат на 42°C, автоматический или полуавтоматический промыватель планшетов (вошер), спектрофотометр (ридер) для микропланшетов на 450/620-695 nm, соответствующие контейнеры для отходов потенциально зараженного материала, таймер, фильтровальная бумага, одноразовые неопудренные перчатки, дезинфицирующие средства.

## 5. ПРЕДОСТЕРЕЖЕНИЕ И ТЕХНИКА БЕЗОПАСНОСТИ

### 5.1. Предостережение

*Перед проведением анализа внимательно ознакомьтесь с инструкцией по применению. Достоверность результата зависит от четкого следования процедуре анализа.*

- не используйте компоненты ИФА-набора после окончания срока годности;
- не используйте во время анализа и не смешивайте компоненты разных серий, компоненты из наборов разных нозологий или реагенты других производителей в сочетании с набором «EQUI HBsAg»;
- не замораживайте ИФА-набор или его компоненты;
- после использования реагента закрывайте каждый флакон своей крышкой;
- во время промывания контролируйте наполнение и полную аспирацию раствора из лунок;
- каждый раз используйте новый наконечник пипетки для внесения образцов или реагентов;
- избегайте попадания прямых солнечных лучей на реагенты ИФА-набора;
- SOLN TMB должен быть бесцветным перед использованием. Если раствор окрашен в синий или желтый цвет, его нельзя использовать. Избегайте контакта SOLN TMB с металлами или ионами металлов. Для работы используйте только чистую, тщательно выполосканную дистиллированной водой посуду;
- не используйте реагенты, цвет которых не соответствует указанному в пункте 4.1;
- ни в коем случае не используйте одну и ту же посуду для рствора коньюгата и SOLN TMB;
- не проводите визуальный учет результатов анализа (без использования ридера);
- дополнительное оборудование, находящееся в непосредственном контакте с биологическим материалом или компонентами набора, считается загрязненным и нуждается в очищении и обеззараживании;

– ИФА-набор предназначен для 96 анализов. Компоненты после использования и остатки неиспользованных компонентов должны быть утилизированы.

### 5.2. Техника безопасности

– все реагенты набора предназначены только для лабораторного профессионального применения в *in vitro* диагностике и могут использоваться только квалифицированным персоналом;
– постановку анализа проводить только в одноразовых неопудреных перчатках и защитных очках;
– не допускается принимать пищу, пить, курить или пользоваться косметикой в комнате проведения теста;
– не пипетировать растворы ртом;
– CONTROL + ИФА-набора «EQUI HBsAg» содержит очищенный поверхностный антиген вируса гепатита В, выделенный с инактивированным прогреванием сыворотки крови человека, в которой не было обнаружено антител к ВИЧ1/2, ВГС и *Treponema pallidum*, однако работать с контролем следует как с потенциально инфекционным материалом;
– CONTROL - ИФА-набора «EQUI HBsAg» протестирован и признан отрицательным на HBsAg и антитела к ВИЧ1/2, ВГС, *Treponema pallidum*, однако обращаться с контролем и исследуемыми образцами следует как с потенциально опасным инфекционным материалом;
– некоторые компоненты набора содержат низкие концентрации вредных веществ и могут вызвать раздражение кожи и слизистых. При попадании SOLN TMB , SOLN STOP и раствора конъюгата на слизистые или кожу, необходимо немедленно промыть пораженное место большим количеством воды;
– в случае разбрызгивания растворов, не содержащих кислоту, например, сывороток, обработать поверхность дезинфицирующим средством, а затем насухо вытереть фильтровальной бумагой. В ином случае кислоту необходимо сначала нейтрализовать раствором бикарбоната натрия, а затем вытереть поверхность, как описано выше.

### 5.3. Инактивация и утилизация отходов

– жидкие отходы необходимо инактивировать, например, раствором перекиси водорода в конечной концентрации 6% в течение 3 часов при комнатной температуре или гипохлоритом натрия в конечной концентрации 5% в течение 30 минут или другими разрешенными дезинфицирующими средствами;
– твердые отходы следует инактивировать путем автоклавирования при температуре стерилизации не меньше 132°С;
– не автоклавируйте растворы, содержащие азид натрия или гипохлорит натрия;
– утилизацию инактивированных отходов проводить в соответствии с действующим национальным законодательством.

## 6. ХРАНЕНИЕ И ТРАНСПОРТИРОВКА

ИФА-набор стабилен в течение срока годности, указанного на этикетке, если его хранить при температуре 2-8°С. Транспортировать набор при температуре 2-8°С. Допускается одноразовая транспортировка при температуре не выше 23°С в течение двух суток.

## 7. РЕКОМЕНДАЦИИ ПО ОТБОРУ, ТРАНСПОРТИРОВКЕ И ХРАНЕНИЮ ОБРАЗЦОВ

Кровь необходимо отбирать из вены в стерильную пробирку. Пробирка должна быть промаркирована с указанием идентификационных данных пациента и даты отбора образца. Цельную кровь до отделения сыворотки можно хранить до 24 часов при температуре 2-8°С, не допуская замораживания.

Сыворотку или плазму крови можно хранить при температуре 2-8°С не более 3 суток. Допускается более продолжительное хранение замороженной сыворотки при температуре -20°С или -70°С. ЗЗамороженные образцы перед использованием следует разморозить и выдержать при комнатной температуре в течение 30 минут. После размораживания образцы следует перемешать для достижения однородности. Избегать повторного замораживания-оттаивания исследуемых образцов. В случае помутнения сыворотки (или плазмы) освобождаются от нерастворенных включений центрифугированием при 3000 об/мин в течение 10-15 минут. Не следует использовать образцы сывороток с выраженной липидемией, гемолизом, а также бактериальным проростом.

Образцы сывороток транспортировать в термоизоляционных контейнерах. Для этого закрытые промаркированные пробирки необходимо поместить в полиэтиленовый пакет, плотно запечатать и положить в центре термоконтейнера. Замороженные хладагенты положить на дно вдоль боковых стенок термоконтейнера и накрыть ими образцы сывороток.

## 8. ПОДГОТОВКА РЕАГЕНТОВ

*Примечание: Перед использованием выдержите все компоненты ИФА-набора при комнатной температуре 18-25°С в течение 30 минут!*

### 8.1. Подготовка планшета ИФА

Для предупреждения конденсации воды в лунках открывайте STRIPS только после выдерживания 30 минут при комнатной температуре. Раскройте вакуумную упаковку, отделите необходимое количество лунок, а остальное сразу же тщательно упакуйте с влагопоглотителем и храните плотно закрытыми на замок zip-lock при температуре 2-8°С. Хранение упакованного таким образом планшета обеспечивает его стабильность в течение 6 месяцев·

### 8.2. Приготовление промывочного раствора

Для приготовления раствора для промывания разведите TWEEN WASH 20x 1:20 (1+19) дистиллированной или деионизированной водой, потом

перемешайте. Например, 5 ml концентрата + 95 ml воды, чего достаточно для 8 лунок. При наличии кристаллов в концентрате раствора для промывания прогрейте флакон при температуре 37°C до полного растворения кристаллов (15–20 минут). Разведенный раствор можно хранить при температуре 2-8°C не более 7 суток.

**8.3. Приготовление раствора конъюгата**

Рабочее разведение конъюгата готовится следующим образом: разведите CONJ 11x (фиолетовый) в чистом флаконе раствором DIL CONJ (розовый) в соотношении 1:11 (то есть, 1+10), раствор окрашивается в фиолетовый цвет. Например, для 8 лунок анализа добавить до 500 µl DIL CONJ 50 µl CONJ 11x. Раствор конъюгата в рабочем разведении стабильный в течение суток при условии хранения при температуре 2-8°C.

## 9. ПРОЦЕДУРА АНАЛИЗА

9.1. Подготовьте необходимое количество лунок для анализа (четыре лунки для контролей и необходимое количество для исследуемых образцов), вставьте их в рамку планшета ИФА. Лунки с контролями обязательно включайте в каждую постановку анализа.

9.2. Заполните схему внесения образцов.

9.3. Приготовьте раствор для промывания в соответствии с пунктом 8.2.

9.4. Приготовьте раствор конъюгата согласно пункту 8.3.

9.5. Внесите в лунки по 100 µl контролей и исследуемых образцов:
CONTROL + – в лунку A1,
CONTROL - – в лунки B1, C1, D1,
в остальные лунки – исследуемые образцы.

9.6. Внесите в лунки по 50 µl раствора конъюгата поверх контролей и исследуемых образцов. Для предотвращения кросконтаминации образцов внесите раствор конъюгата, не касаясь содержания лунок. Осторожно постукивая по планшету, перемешайте смесь в лунках.

9.7. Заклейте стрипы клейкой пленкой и инкубируйте в течение 120 минут при 37°C и постоянном орбитальном перемешивании содержимого лунок со скоростью 300 об/мин. *Инкубацию образцов с конъюгатом в лунках ИФА-планшета можно проводить в течение 120 минут при температуре 42°C в статическом режиме. Однако при этом может наблюдаться снижение специфичности анализа.*

9.8. По окончании инкубации аккуратно снимите клейкую пленку и промойте лунки пять раз с использованием автоматического промывателя или 8-канальной пипетки следующим образом:
– удалите содержимое лунок в контейнер для жидких отходов;
– наполните лунки стрипов не менее чем по 300 µl раствором для промывания, оставьте не менее, чем на 30 секунд;
– аспирируйте раствор из лунок. Остаточный объем раствора после каждого этапа аспирации должен составлять не больше 5 µl;

– повторите процедуру промывания еще пять раз;
– после последней аспирации избавьтесь от лишней влаги, постукивая планшетом по фильтровальной бумаге.

9.9. Внесите в лунки по 100 µl SOLN|TMB, не касаясь дна и стенок лунок планшета.

9.10. Инкубируйте стрипы в течение 30 минут в темном месте при комнатной температуре 18-25°C. Не используйте клейкую пленку на данном этапе.

9.11. Внесите в лунки стрипов по 100 µl SOLN|STOP для остановки ферментативной реакции, придерживаясь той же последовательности, что и при внесении SOLN|TMB. Во время внесения происходит изменение цвета раствора с голубого на желтый, в лунках с прозрачным раствором незначительно меняется оттенок.

9.12. Измерьте на ридере ОП в каждой лунке при длине волны 450/620-695 nm в течение 5 минут после остановки реакции. До проведения измерения убедитесь в чистоте внешней поверхности дна лунок и отсутствии пузырьков.

*Учет результатов анализа можно проводить в одноволновом режиме при длине волны 450 nm, в этом случае оставьте лунку для установления бланка (в такую лунку внесите только SOLN|TMB и SOLN|STOP).*

## 10. УЧЁТ РЕЗУЛЬТАТОВ И ИХ ИНТЕРПРЕТАЦИЯ

### 10.1. Учет результатов анализа

Рассчитать среднее значение ОП негативного контроля ($\overline{Nc}$) уровень граничного значения (Cut off - CO).

$$\overline{Nc} = (Nc1 + Nc2 + Nc3)/3; \quad CO = \overline{Nc} + 0{,}07$$

### 10.2. Контроль достоверности результатов анализа

Данные теста считаются достоверными, если они соответствуют следующим требованиям:

| | | |
|---|---|---|
| CONTROL + | ОП ≥ 1,5 | |
| CONTROL - | ОП ≤ 0,100 | |
| CONTROL - | $\overline{Nc} \times 0{,}5 \le Ncn \le \overline{Nc} \times 2{,}0$ | где Ncn – ОП каждого повтора Nc |

Если одно из значений ОП негативного контроля выходит за пределы указанного выше интервала, его отбрасывают и рассчитываю $\overline{Nc}$ по остальным значениям ОП негативного контроля. Если более одного значения ОП негативного контроля не отвечает указанным требованиям, то тест считается некорректным и требует повторного проведения.

### 10.3. Интерпретация результатов

$OD_{sample} \geq CO$ ПОЛОЖИТЕЛЬНЫЙ

$OD_{sample} < CO$ ОТРИЦАТЕЛЬНЫЙ** , где $OD_{sample}$ – ОП образца

* Первоначально положительные образцы должны быть исследованы повторно в двух лунках ИФА-набора «EQUI HBsAg». После повторного тестирования положительными считаются образцы, оптическая плотность которых хотя бы в одном из повторов превышает граничное значение. Если при повторном тестировании оптическая плотность образца в обоих повторах ниже граничного значения, такой образец считать отрицательным.

Результаты для образцов, ОП которых равно граничному значению или находится в пределах ±10%, следует интерпретировать осторожно. Такие образцы должны быть исследованы повторно в двух лунках набора «EQUI HBsAg». Если при повторном тестировании $OD_{sample}$ снова находится в пределах ±10% граничного значения, следует провести отбор и анализ нового образца.

** Образцы со значением оптической плотности ниже граничного значения считаются отрицательными в ИФА-наборе «EQUI HBsAg». Однако результаты в пределах 10% ниже граничного значения следует интерпретировать с осторожностью (рекомендуется повторно исследовать такие образцы в двух лунках набора ИФА).

## 11. ХАРАКТЕРИСТИКИ ТЕСТА

### 11.1. Аналитические характеристики

**Прецизионность**

*Воспроизводимость результатов в пределах одной постановки анализа (Intra assay repeatability)*

Коэффициент вариации (CV) для двух сывороток с разной концентрацией поверхностного антигена оценивали в 32 повторах на одной серии ИФА-наборов.

| № сыровотки | $ОП_{ср}$ | CV, % |
|---|---|---|
| 2 | 1,809 | 3,3 |
| 45/15 | 0,922 | 3,7 |

*Воспроизводимость результатов между разными постановками анализа (Inter assay reproducibility)*

Коэффициент вариации (CV) для двух сывороток с разным уровнем специфических антител оценивали в течение 4 дней в 4 постановках анализа по 8 повторов в каждом анализе.

| № сыровотки | $ОП_{ср}$ | CV, % |
|---|---|---|
| 2 | 1,827 | 5,6 |
| 45/15 | 0,936 | 5,8 |

**Аналитическая чувствительность**

Предел чувствительности анализа по обнаружению поверхностного антигена вируса гепатита В определяли на Британском стандартном образце 07/288-010 для HBsAg (Национальный институт биологических стандартов Соединенного королевства, NIBSC) и подтверждали с использованием Третьего Международного Стандарта для HBsAg 12/226 (Third International Standard for HBsAg, производства NIBSC). Предел чувствительности ИФА-набора «EQUI HBsAg» составил 0,05 IU/ml (МЕ/мл).

**Аналитическая специфичность**

На результат анализа не влияет присутствие в образце билирубина в концентрации до 0,1 mg/ml (172,3 µmol/l), гемоглобина в концентрации до 5 mg/ml и триглицеридов в концентрации до 10 mg/ml (11,3 mmol/ l).

**11.2. Диагностические характеристики**

Для определения клинической чувствительности и специфичности наборов «EQUI HBsAg» использовали 57 образцов сывороток, полученных от пациентов с диагнозом гепатит В, и 294 образца сывороток клинически здоровых доноров (сероотрицательных по отношению к вирусу гепатита В). Кроме того, были использованы образцы из коммерческих панелей производства «SeraCare Life Sciences» (США). По результатам анализа клиническая чувствительность ИФА-набора составляет 100%, клиническая специфичность – 100%.

Исследование характеристик метода по сравнению с аналогичной коммерческой тест-системой проводилось на целевой группе беременных женщин (171 образец). Для выборки беременных женщин относительная специфичность составляла 100%, процент совпадения – 100%.

Положительная прогностическая ценность (PPV) ИФА-набора «EQUI HBsAg» составляет 100%, отрицательная прогностическая ценность (NPV) – 100%.

## 12. ОГРАНИЧЕНИЕ АНАЛИЗА

Отрицательный результат в ИФА-наборе «EQUI HBsAg» показывает, что тестируемый образец не содержит HBsAg или его концентрация ниже 0,05 IU/ml (МЕ/мл). Поскольку образец может содержать HBsAg в очень низкой концентрации, отрицательный результат в ИФА-наборе «EQUI HBsAg» не позволяет полностью исключить инфицирование вирусом гепатита В.

Кроме того, в литературных источниках описаны некоторые примеры вирусного гепатита В (острого или хронического), когда в образце обнаруживалась вирусная ДНК при отсутствии HBsAg. В таких случаях полезным будет исследование образца на другие маркеры вирусного гепатита В, выявление ДНК и оценка биохимических показателей сыворотки крови пациента.

Для верификации специфичности реакции каждый положительный результат (согласно критериям интерпретации ИФА-набора «EQUI HBsAg») необходимо подтвердить в нейтрализационном ИФА с использованием комплекта реагентов «EQUI HBsAg Confirmation». Для корректной диагностики гепатита В рекомендуется провести исследование образца на наличие специфических антител классов IgM и IgG к HBcore антигену и антител к HBsAg (например, в ИФА-наборах «EQUI HBcore IgM», «EQUI HBcore IgG» и «EQUI anti-HBs», соответственно).

В целях нивелирования ложноположительных результатов, вызванных наличием в образцах сывороток крови человека антител, специфических к иммуноглобулинам мыши, в ИФА-наборе используется специальный блок-компонент, препятствующий формированию иммунных комплексов с антимышиными антителами (англ. HAMA) на твердой фазе.

## 13. ТРУДНОСТИ, КОТОРЫЕ МОГУТ ВОЗНИКНУТЬ ПРИ ПРОВЕДЕНИИ ИФА

***Высокий фон в лунках всего планшета может возникнуть из-за:***

– загрязненного промывателя;
– низкого качества или загрязнения воды;
– использования плохо помытой посуды;
– использования дезинфицирующих средств, содержащих хлор;
– использования загрязненных наконечников;
– увеличения времени инкубации или изменения температурного режима.

***Высокий фон в отдельных рядах может быть связан с:***

– повторным внесением раствора ТМБ;
–загрязнением конуса автоматической пипетки раствором конъюгата;
– загрязнением одного из каналов промывателя.

***Полученное значение ОП положительного контроля ниже установленной границы, если***:

– неправильно приготовлен или не внесен один из реагентов (раствор конъюгата или раствор ТМБ);
– сокращено время инкубации на одном из этапов.

***Интенсивность окрашивания лунок не соответствует полученной оптической плотности.*** Это может свидетельствовать о смещенном оптическом луче.

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 Производитель

IVD Медицинское изделие для диагностики *in vitro*

REF Номер по каталогу

 Дата изготовления

 Использовать до

LOT Код партии

 Температурное ограничение

 Содержит достаточно для (n-) испытаний

 Предостережение, ознакомиться с сопроводительными документами

 Ознакомление с инструкцией по применению

 Беречь от прямых солнечных лучей

 Знак соответствия техническим регламентам

Редакция 8 от 21.09.2021г.

*С вопросами и пожеланиями по работе набора обращайтесь к производителю:*

ООО «Эквитестлаб»
ул. Большая Васильковская 114, г. Киев, Украина, 03150

проспект Победы 60/2, г. Киев, Украина, 03057
(адрес производства)

тел.: 0 (800)31-89-87, +38 (044)334-89-87,
e-mail: info@equitest.com.ua, www.equitest.com.ua

## СХЕМА ПРОВЕДЕНИЯ АНАЛИЗА

| |
|---|
| Выдержать реагенты 30 min при температуре 18-25°C |
| Внести по 100 µl контролей и исследуемых образцов в лунки:<br>A1 – CONTROL +, B1, C1, D1 – CONTROL −,<br>E1 и в остальные лунки - исследуемые образцы |
| В лунки стрипов внести по 50 µl приготовленного 1:11 (1+10) раствора конъюгата.<br>(фиолетовый цвет) |
| Заклеить стрипы пленкой, инкубировать **120 мин при температуре 37°C** и постоянном орбитальном перемешивании содержимого лунок со скоростью 300 об/мин |
| Промыть лунки 6 раз приготовленным 1:20 (1+19) промывным раствором TWEEN (300 µl в лунку) |
| В лунки стрипов внести по 100 µl SOLN TMB |
| Инкубировать на протяжении **30 min в темноте при температуре 18-25°C** |
| В лунки стрипов внести по 100 µl SOLN STOP<br>(происходит изменение цвета с голубого на желтый) |

## УЧЕТ РЕЗУЛЬТАТОВ АНАЛИЗА

$\overline{Nc}$ = (Nc1 + Nc2 + Nc3)/3;

CO = $\overline{Nc}$ + 0,07;

$\overline{Nc}$ - Среднее значение ОП 3-х CONTROL −

CO - Уровень граничного значения (Cut off)

## ИНТЕРПРЕТАЦИЯ РЕЗУЛЬТАТОВ

| | |
|---|---|
| $OD_{sample} \geq CO$ | ПОЛОЖИТЕЛЬНЫЙ |
| $OD_{sample} < CO$ | ОТРИЦАТЕЛЬНЫЙ |

# CERTIFICATE

**MANAGEMENT SYSTEM CERTIFICATION BODY**
**«CONFORMITY ASSESSMENT BODY «PROMSTANDART», LLC**
certifies that the enterprise

## EKVITESTLAB
**Limited Liability Company**

**registration code 38745936**

**legal address:**
**Ukraine, 03150, Kyiv, 114 Velyka Vasylkivska street,**

manufacturer's address:
Ukraine, 04212, Kyiv, 60/2 Peremohy Avenue

has established and applies quality management system for
**development, production, storage and sale of ELISA kits for in vitro diagnostic**

Audit, № report 2020/015-20.2.1
confirmed that the requirements

## ISO 13485:2016
**«Medical devices — Quality management systems — Requirements for regulatory purposes»**

are performed.

The control of conformity of the certified quality management system to the requirements of the specified standard is carried out by means of supervisory audit, the periodicity and procedures of which are regulated by the program.

| | |
|---|---|
| **Certificate registration number** | **№ UA.QMS.00014-21** |
| **Registered** | **06 April 2021** |
| **Valid until** | **05 April 2024** |

8O156
DSTU EN ISO/IEC 17021-1

**Director of Certification Body**
**«CAB «PROMSTANDART», LLC**

stamp (signature)

**Sergiy Dubrovskyi**

210107

The validity of certificate can be verified by telephone: (056) 742-82-39
or on website of «CAB «PROMSTANDART», LLC: prom-standart.com.ua

ПРОМСТАНДАРТ ■ PROMSTANDART ■ ПРОМСТАНДАРТ ■ PROMSTANDART ■ ПРОМСТАНДАРТ

浙江东方基因生物制品股份有限公司
Zhejiang Orient Gene Biotech Co., LTD

CE-DOC-OG038
Version 2.0

# EC Declaration of Conformity

In accordance with Directive 98/79/EC

**Legal Manufacturer:** *Zhejiang Orient Gene Biotech Co., Ltd*

**Legal Manufacturer Address:** *3787#, East Yangguang Avenue, Dipu Street, Anji 313300, Huzhou, Zhejiang, China*

Declares, that the products
Product Name and Model(s)

| | |
|---|---|
| Troponin I Rapid Test Cassette (Whole Blood/Serum/Plasma) | GDTRO-402a |
| Troponin I Rapid Test Cassette (Whole Blood/Serum/Plasma) | GDTRO-402b |

Classification: *Other*
Conformity assessment route: *Annex III (EC DECLARATION OF CONFORMITY)*

We, the Manufacturer, herewith declare with sole responsibility that our product/s mentioned above meet/s the provisions of the Directive 98/79/EC of the European Parliament and of the Council on In-Vitro Diagnostic Medical Devices.

We hereby explicitly appoint

**EC Representative's Name:** Shanghai International Holding Corp. GmbH (Europe)

**EC Representative's Address:** Eiffestrasse 80, 20537 Hamburg, Germany

to act as our European Authorized Representative as defined in the aforementioned Directive.

I, the undersigned, hereby declare that the medical devices specified above conform with the directive 98/79/EC on in vitro diagnostic medical devices and pertinent essential requirements

Date Signed: August 11, 2020

Name of authorized signatory: Joyce Pang
Position held in the company: Vice-President

浙江东方基因生物制品股份有限公司
Zhejiang Orient Gene Biotech Co., LTD

CE-DOC-OG048
Version 3.0

CE

# EC Declaration of Conformity

In accordance with Directive 98/79/EC

**Legal Manufacturer:** *Zhejiang Orient Gene Biotech Co., Ltd*

**Legal Manufacturer Address:** *3787#, East Yangguang Avenue, Dipu Street, Anji 313300, Huzhou, Zhejiang, China*

Declares, that the products
Product Name and Model(s)

| | |
|---|---|
| D-Dimer Rapid Test Cassette (Whole Blood/Plasma) | GDDDI-402b |

Classification: Other
Conformity assessment route: *Annex III (EC DECLARATION OF CONFORMITY)*

We, the Manufacturer, herewith declare with sole responsibility that our product/s mentioned above meet/s the provisions of the Directive 98/79/EC of the European Parliament and of the Council on In-Vitro Diagnostic Medical Devices.

We hereby explicitly appoint

**EC Representative's Name:** QARAD BV

**EC Representative's Address:** Cipalstraat 3, 2440 Geel BELGIUM

to act as our European Authorized Representative as defined in the aforementioned Directive.

I, the undersigned, hereby declare that the medical devices specified above conform with the directive 98/79/EC on in vitro diagnostic medical devices and pertinent essential requirements

Date Signed: November 11, 2021

Joyce Pang

Name of authorized signatory: Joyce Pang
Position held in the company: Vice-President

浙江东方基因生物制品股份有限公司
Zhejiang Orient Gene Biotech Co., LTD

CE-DOC-OG060
Version 1.0

# EC Declaration of Conformity

In accordance with Directive 98/79/EC

**Legal Manufacturer:** *Zhejiang Orient Gene Biotech Co., Ltd*

**Legal Manufacturer Address:** *3787#, East Yangguang Avenue, Dipu Street, Anji 313300, Huzhou, Zhejiang, China*

Declares, that the products
Product Name and Model(s)

| | |
|---|---|
| Fecal Occult Blood Rapid Test Strip (Feces) | GEFOB-601b |
| Fecal Occult Blood Rapid Test Cassette (Feces) | GEFOB-602b |

Classification: *Other*
Conformity assessment route: *Annex III (EC DECLARATION OF CONFORMITY)*

We, the Manufacturer, herewith declare with sole responsibility that our product/s mentioned above meet/s the provisions of the Directive 98/79/EC of the European Parliament and of the Council on In-Vitro Diagnostic Medical Devices.

We hereby explicitly appoint

**EC Representative's Name:** Shanghai International Holding Corp. GmbH (Europe)

**EC Representative's Address:** Eiffestrasse 80, 20537 Hamburg, Germany

to act as our European Authorized Representative as defined in the aforementioned Directive.

I, the undersigned, hereby declare that the medical devices specified above conform with the directive 98/79/EC on in vitro diagnostic medical devices and pertinent essential requirements

Date Signed: November 28, 2017

Name of authorized signatory: Joyce Pang
Position held in the company: Vice-President

ZERTIFIKAT ◆ CERTIFICATE ◆ 認證證書 ◆ СЕРТИФИКАТ ◆ CERTIFICADO ◆ CERTIFICAT

DAkkS Deutsche Akkreditierungsstelle D-ZM-11321-01-00

Product Service

# Certificate

## No. Q5 092305 0001 Rev. 01

**Holder of Certificate:** **Zhejiang Orient Gene Biotech Co., Ltd.**
3787#, East Yangguang Avenue, Dipu Street Anji
313300 Huzhou, Zhejiang
PEOPLE'S REPUBLIC OF CHINA

**Certification Mark:**

**Scope of Certificate:** **Design and Development, Production and Distribution of In Vitro Diagnostic Reagent and Instrument for the Detection of Drugs of Abuse, Fertility, Infectious Diseases, Oncology, Biochemistry, Cardiac Diseases, Allergic Disease based on Rapid Test, PCR and Liquid Biochip Method.**

The Certification Body of TÜV SÜD Product Service GmbH certifies that the company mentioned above has established and is maintaining a quality management system, which meets the requirements of the listed standard(s). All applicable requirements of the testing and certification regulation of TÜV SÜD Group have to be complied with. For details and certificate validity see: www.tuvsud.com/ps-cert?q=cert:Q5 092305 0001 Rev. 01

**Report No.:** SH2198802

**Valid from:** 2022-04-11
**Valid until:** 2024-03-16

**Date,** 2022-04-11

Christoph Dicks
Head of Certification/Notified Body


TÜV SÜD Product Service GmbH • Certification Body • Ridlerstraße 65 • 80339 Munich • Germany

ZERTIFIKAT ◆ CERTIFICATE ◆ 認證證書 ◆ СЕРТИФИКАТ ◆ CERTIFICADO ◆ CERTIFICAT

DAkkS Deutsche Akkreditierungsstelle D-ZM-11321-01-00

TÜV SÜD Product Service

# Certificate

## No. Q5 092305 0001 Rev. 01

**Applied Standard(s):**

EN ISO 13485:2016
Medical devices - Quality management systems - Requirements for regulatory purposes
(ISO 13485:2016)
DIN EN ISO 13485:2016

**Facility(ies):**

Zhejiang Orient Gene Biotech Co., Ltd.
3787#, East Yangguang Avenue, Dipu Street Anji, 313300
Huzhou, Zhejiang, PEOPLE'S REPUBLIC OF CHINA

See Scope of Certificate

.


TÜV SÜD Product Service GmbH • Certification Body • Ridlerstraße 65 • 80339 Munich • Germany

TÜV®

# STATEMENT

We, Zhejiang Orient Gene Biotech Co., Ltd , having a registered office at 3787#, East Yangguang Avenue, Dipu Street Anji 313300, Huzhou, Zhejiang, China assign SRL SANMEDICO having a registered office at A. Corobceanu street 7A, apt. 9, Chişinău MD-2012, Moldova, as non-exclusive authorized representative for Orient Gene Brand product in correspondence with the conditions of directive 98/79/EEC.

We declare that the company mentioned above is authorized to register, notify, renew or modify the registration of medical devices on the territory of the Republic of Moldova.

This Statement letter will be valid from Feb.21th,2023 to Feb.20th, 2024.

Zhejiang Orient Gene Biotech Co., Ltd

General Manager:

Date:2023/2/21

地址：浙江省湖州市安吉县递铺镇阳光大道东段 3787 号
Add: **3787#, East Yangguang Avenue, Dipu Street Anji 313300, Huzhou, Zhejiang, China**
电话 Tel:+86-572-5226111　　传真 Fax: +86-572-5226222　邮编 P.C.:313300

# Troponin I

## Troponin I Rapid Test Device (Whole Blood/Serum/Plasma) Package Insert

*A rapid visual immunoassay for the qualitative presumptive detection of cardiac Troponin I in human whole blood, serum, or plasma specimens.*
*For professional in vitro diagnostic use only.*

**INTENDED USE**

The Troponin I Rapid Test Device (Whole Blood/Serum/Plasma) is a rapid visual immunoassay for the qualitative presumptive detection of cardiac Troponin I in human whole blood, serum, or plasma specimens. This kit is intended to be used as an aid in the diagnosis of myocardial infarction (MI).

**SUMMARY**

Cardiac Troponin I (cTnI) is a protein found in cardiac muscle with a molecular weight of 22.5 kDa.[1] Troponin I is part of a three subunit complex comprising of Troponin T and Troponin C. Along with tropomyosin, this structural complex forms the main component that regulates the calcium sensitive ATPase activity of actomyosin in striated skeletal and cardiac muscle.[2] After cardiac injury occurs, Troponin I is released into the blood 4-6 hours after the onset of pain. The release pattern of cTnI is similar to CK-MB, but while CK-MB levels return to normal after 72 hours, Troponin I remains elevated for 6-10 days, thus providing for a longer window of detection for cardiac injury. The high specificity of cTnI measurements for the identification of myocardial damage has been demonstrated in conditions such as the perioperative period, after marathon runs, and blunt chest trauma.[3] cTnI release has also been documented in cardiac conditions other than acute myocardial infarction (AMI) such as unstable angina, congestive heart failure, and ischemic damage due to coronary artery bypass surgery.[4] Because of its high specificity and sensitivity in the myocardial tissue, Troponin I has recently become the most preferred biomarker for myocardial infarction.[5]

**PRINCIPLE**

The Troponin I Rapid Test Device (Whole Blood/Serum/Plasma) has been designed to detect cardiac Troponin I through visual interpretation of color development in the strip. The membrane was immobilized with anti-cTnI antibodies on the test region.
During the test, the specimen is allowed to react with colored anti-cTnI antibodies colloidal gold conjugates, which were precoated on the sample pad of the test. The mixture then moves on the membrane by a capillary action, and interact with reagents on the membrane. If there were enough cTnI in specimens, a colored band will form at the test region of the membrane.
Presence of this colored band indicates a positive result, while its absence indicates a negative result. Appearance of a colored band at the control region serves as a procedural control. This indicates that proper volume of specimen has been added and membrane wicking has occurred.

**PRECAUTIONS**

1. For professional In Vitro diagnostic use only.
2. Warning: the reagents in this kit contain sodium azide which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
3. Do not use it if the tube/pouch is damaged or broken.
4. Test is for single use only. Do not re- use under any circumstances.
5. Handle all specimens as if they contain infectious agents. Observe established standard procedure for proper disposal of specimens
6. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assay.
7. Humidity and temperature can adversely affect results

**STORAGE AND STABILITY**

All reagents are ready to use as supplied. Store unused test device unopened at 2°C-30°C. If stored at 2°C-8°C, ensure that the test device is brought to room temperature before opening. The test is not stable out off the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit over 30°C.

**SPECIMEN COLLECTION AND PREPARATION**

- The Troponin I Rapid Test Device (Whole Blood/Serum/Plasma) is intended only for use with human whole blood, serum, or plasma specimens.
- Only clear, non-hemolyzed specimens are recommended for use with this test.
- Serum or plasma should be separated with soonest possible opportunity to avoid hemolysis.
- Perform the testing immediately after the specimen collection. Do not leave the specimens at room temperature for prolonged periods. Specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
- Pack the specimens in compliance with applicable regulations for transportation of etiological agents, in case they need to be shipped.
- Icteric, lipemic, hemolysed, heat treated and contaminated sera may cause erroneous results.
- There is a slight possibility that some whole blood specimens with very high viscosity or which have been stored for more than 2 days may not run properly on the test device. Repeat the test with a serum or plasma specimen from the same patient using a new test device.

**MATERIALS**

**Materials Provided**

- Test devices
- Buffer
- Disposable Droppers
- Package insert

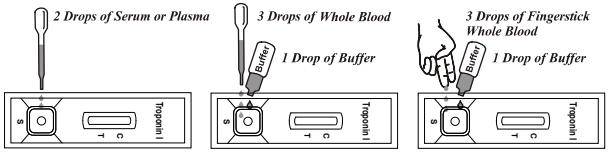
**Materials Required But Not Provided**

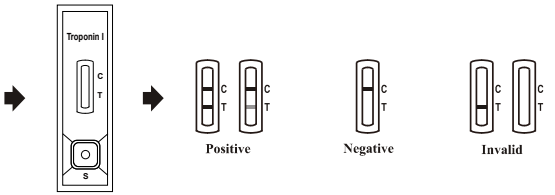
- Specimen collection containers
- Centrifuge (for plasma only)
- Clock or Timer

**DIRECTIONS FOR USE**

**Allow test device, specimen, buffer and/or controls to equilibrate to room temperature (15-30°C) prior to testing.**

1. Remove the test from its sealed pouch, and place it on a clean, level surface. Label the device with patient or control identification. To obtain a best result, the assay should be performed within one hour.
2. Transfer 2 drops of serum or plasma to the specimen well of the device with a disposable pipette provided in the kit, and then start the timer.
   **OR**
   Transfer 3 drops of whole blood specimen to the specimen well of the device with a disposable pipette provided in the kit, then add 1 drop of buffer, and start the timer.
   **OR**
   Allow 3 hanging drops of fingerstick whole blood specimen to fall into the center of the specimen well (S) on the device, then add 1 drop of buffer, and start the timer. Avoid trapping air bubbles in the specimen well (S), and do not drop any solution in observation window.
   As the test begins to work, you will see color move across the membrane.
3. Wait for the colored band(s) to appear. The result should be read at 10 minutes. Do not interpret the result after 20 minutes.




**INTERPRETATION OF RESULTS**

(Please refer to the illustration above)
**POSITIVE**:Two colored bands appear on the membrane. One band appears in the control region (C) and another band appears in the test region (T).
**NEGATIVE**:Only one colored band appears in the control region (C). No apparent colored band appears in the test region (T).
**INVALID**:Control band fails to appear. Results from any test which has not produced a control band at the specified reading time must be discarded.
Please review the procedure and repeat with a new test. If the problem persists, discontinue using the kit immediately and contact your local distributor.

**NOTE:**

1. The intensity of the color in test region (T) may vary depending on the concentration of aimed substances present in the specimen. Therefore, any shade of color in the test region should be considered positive. Besides, the substances level can not be determined by this qualitative test.
2. Insufficient specimen volume, incorrect operation procedure, or performing expired tests are the most likely reasons for control band failure.

**QUALITY CONTROL**

Internal procedural controls are included in the test. A colored band appearing in the control region (C) is considered an internal positive procedural control. It confirms sufficient specimen volume and correct procedural technique.

External controls are not supplied with this kit. It is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

**LIMITATIONS**

1. The Troponin I Rapid Test Device (Whole Blood/Serum/Plasma) is for professional in vitro diagnostic use, and should be used for the qualitative detection of cardiac Troponin I only. There is no meaning attributed to linen color intensity or width.
2. The Troponin I Rapid Test Device (Whole Blood/Serum/Plasma) will only indicate the presence of Troponin I in the specimen and should not be used as the sole criteria for the diagnosis of tuberculosis.
3. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. The test cannot detect less than 0.5 ng/mL of cTnI in specimens. Thus, a negative result does not at anytime rule out the existence of Troponin I in blood, because the antibodies may be absent or below the minimum detection level of the test.
4. Like with all diagnostic tests, a confirmed diagnosis should only be made by a physician after all clinical and laboratory findings have been evaluated.
5. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor (RF) may affect expected results. Even if the test results are positive, further clinical evaluation should be considered with other clinical information available to the physician.

**PERFORMANCE CHARACTERISTICS**

Table: Troponin I Rapid Test vs. EIA

| Method | | Troponin I Rapid Test Device | | Total Results |
|---|---|---|---|---|
| EIA | Results | Positive | Negative | |
| | Positive | 138 | 2 | 140 |
| | Negative | 1 | 315 | 316 |
| Total Results | | 139 | 317 | 456 |

Relative Sensitivity: 98.6% (94.9%-99.8%)*
Relative Specificity: 99.7% (98.3%-99.9%)*
Overall Agreement: 99.3% (98.1%-99.9%)*
*95% Confidence Interval

**BIBLIOGRAPHY**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5. Alpert JS, et al. Myocardial Infarction Redefined, Joint European Society of Cardiology American College of Cardiology: J. Am. Coll. Cardio., 36(3):959, 2000.

# Fecal Occult Blood Rapid Test Cassette (Feces) CE

## INTENDED USE

Fecal Occult Blood Rapid Test Cassette (Feces) is a rapid chromatographic immunoassay for the qualitative detection of human occult blood in feces by professional laboratories or physician's offices. It is useful to detect bleeding caused by a number of gastrointestinal disorders, e.g., diverticulitis, colitis, polyps, and colorectal cancer.
Fecal Occult Blood Rapid Test Cassette (Feces) is recommended for use in1) routine physical examinations, 2) hospital monitoring for bleeding in patients, and 3) screening for colorectal cancer or gastrointestinal bleeding from any source.

## INTRODUCTION

Most of diseases can cause hidden blood in the stool. In the early stages, gastrointestinal problems such as colon cancer, ulcers, polyps, colitis, diverticulitis, and fissures may not show any visible symptoms, only occult blood. Traditional guaiac-based method lacks sensitivity and specificity, and has diet-restriction prior to the testing.
Fecal Occult Blood Rapid Test Cassette (Feces) is a rapid test to qualitatively detect low levels of fecal occult blood in feces. The test uses double antibod- sandwich assay to selectively detect as low as 50 ng/mL of hemoglobin or 6 µg hemoglobin/g feces. In addition, unlike the guaiac assays, the accuracy of the test is not affected by the diet of the patients.

## PRINCIPLE

Fecal Occult Blood Rapid Test Cassette (Feces) is a lateral flow chromatographic immunoassay based on the principle of the double antibody-sandwich technique. The membrane is pre-coated with anti-hemoglobin antibodies on the test line region of the device. During testing, the specimen reacts with the colloidal gold coated withl anti-hemoglobin antibodies. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-hemoglobin antibodies on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

## MATERIALS PROVIDED

20 Test cassettes
20 Specimen collection tubes with buffer
1 Package insert

## MATERIALS REQUIRED BUT NOT PROVIDED

1. Specimen collection containers 2. Clock or timer

## STORAGE AND STABILITY

All reagents are ready to use as supplied. Store unused test device unopened at 2°C-30°C. If stored at 2°C-8°C, ensure that the test device is brought to room temperature before opening. The test is not stable out of the expiration date printed on the sealed pouch. Do not freeze the kit or expose the kit over 30°C.

## PRECAUTIONS

1. For professional *in vitro* diagnostic use only.
2. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
3. Do not use it if the tube/pouch is damaged or broken.
4. Test is for single use only. Do not re-use under any circumstances.
**5. Do not use specimen with visible blood for the testing.**
6. Handel all specimens as if they contain infectious agents. Observe established standard procedure for proper disposal of specimens.
7. Specimen extraction buffer contains Sodium Azide (0.1%). Avoid contact with skin or eyes. Do not ingest.
8. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assay.
9. Humidity and temperature can adversely affect results.
10. Do not perform the test in a room with strong air flow, ie. electric fan or strong airconditioning.

## PATIENT PREPARATION

1. A specimen should not be collected from a patient with following conditions that may interfere with the test results:

- Menstrual bleeding
- Bleeding hemorrhoids
- Constipating bleeding
- Urinary bleeding.

2. Dietary restrictions are not necessary.
3. Alcohol and certain medications such as aspirin, indomethacin, phenylbutazone, reserpine, cortocosteroids, and nonsteroidal anti-inflammatory drugs may cause gastrointestinal irritation and subsequent bleeding, thus gives positive reactions. On the advice of the physician, such substances should be discontinued at least 48 hours prior to testing.

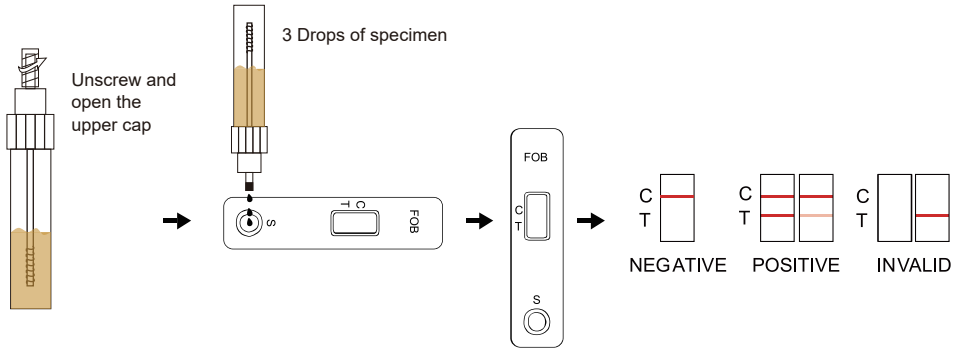
## SPECIMEN COLLECTION AND PREPARATION

Consider any materials of human origin as infectious and handle them using standard biosafety procedures.
1. Collect a random sample of feces in a clean, dry receptacle.
2. Unscrew the top of the collection tube and remove the applicator stick.
3. Randomly pierce the fecal specimen in at least five (5) different sites.
4. Remove excess sample off the shaft and outer grooves. Be sure sample remains on inside grooves.
5. Replace the stick in the tube and tighten securely.
6. Shake the specimen collection bottle so that there is proper homogenisation of feces in buffer solution.

***Note:*** Specimens prepared in the specimen collection tube may be stored at room temperature (15-30°C) for 3 days maximum, at 2-8°C for 7 days maximum or at -20°C for 3 months maximum if not tested within 1 hour after preparation.

## TEST PROCEDURE

**Allow the test cassette, specimen, and/or controls to reach room temperature (15-30°C) prior to testing.**
1. Remove the test cassette from the foil pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
2. Place the test cassette on a clean, flat surface.
3. Shake the specimen collection tube several times.
4. Hold the specimen collection tube upright and then unscrew and open the upper cap.
5. Squeeze 3 drops (~90 µL) of the sample solution in the sample well of the cassette and start the timer.
6. Wait for the colored line(s) to appear. Read results in 5 minutes. Do not interpret the result after 5 minutes.




## INTERPRETATION OF RESULTS

(Please refer to the illustration above)
**Positive:** Two lines appear. One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T).
**Negative:** One colored line appears in the control line region(C). No line appears in the test line region (T).
**Invalid:** Control line fails to appear. The test should be repeated using a new cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.
***NOTE:***
1. The intensity of color in the test region (T) may vary depending on the concentration of analytes present in the specimen. Therefore, any shade of color in the test region should be considered positive. Note that this is a qualitative test only, and

# Fecal Occult Blood Rapid Test Cassette (Feces)

cannot determine the concentration of analytes in the specimen.
2. Insufficient specimen volume, incorrect operating procedure or expired tests are the most likely reasons for control band failure.

## QUALITY CONTROL

An internal procedural control is included in the test. A colored line appearing in the control line region (C) is an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correctl procedural technique. Control standards are not supplied with this kit; however it is recommended that positive and negative controls be tested as a good laboratory practice to confirm the test procedure and to verify proper test performance.

## LIMITATIONS

1. This test kit is to be used for the qualitative detection of human hemoglobin in fecal samples. A positive result suggests the presence of human hemoglobin in fecal samples. In addition to intestinal bleeding the presence of blood in stools may have other causes such as hemorrhoids, blood in urine etc.
2. Not all colorectal bleedings are due to precancerous or cancerous polyps. The information obtained by this test should be used in conjunction with other clinical findings and testing methods, such as colonoscopy gathered by the physician.
3. Negative results do not exclude bleeding since some polyps and colorectal region cancers can bleed intermittently or not at all. Additionally, blood may not be uniformly distributed in fecal samples. Colorectal polyps at an early stage may not bleed.
4. Urine and excessive dilution of sample with water from toilet bowl may cause erroneous test results. The use of a receptacle is recommended.
5. Feces specimens should not collect during the menstrual period and not three day before or afterwards, at bleeding due to constipation, bleeding haemorrhoids,or at taking rectally administered medication. It could cause false positive results.
6. This test may be less sensitive for detecting upper g.i. Bleeding because blood degrades as it passes through the g.i. Track.
7. The Fecal Occult Blood Rapid Test Cassette (Feces) is to aid indiagnosis and is not intended to replace other diagnostic procedures such as G.I. fibroscope, endoscopy, colonoscopy, or X-ray analysis. Test results should not be deemed conclusive with respect to the presence or absence of gastrointestinal bleeding or pathology. A positive result should be followed up with additional diagnostic procedures to determine the exact cause and source for the occult blood in the feces.

## PERFORMANCE CHARACTERISTICS

**1. Sensitivity:** 99.6%
Fecal Occult Blood Rapid Test Cassette (Feces) can detect the levels of human occult blood as low as 50 ng/mL hemoglobin or 6 µg hemoglobin/g feces.
**2. Prozone Effect:**
It is observed that this FOB test can detect 2 mg/mL hemoglobin.
**3. Specificity:** 99.9%
Fecal Occult Blood Rapid Test Cassette (Feces) is specific to human hemoglobin. Specimen containing the following substances at the standard concentration was tested on both positive and negative controls and showed no effects on test results at standards concentration.

| Substances | Concentrations (Diluted with the extraction buffer) |
|---|---|
| Beef hemoglobin | 2 mg/mL |
| Chicken hemoglobin | 0.5 mg/mL |
| Pig hemoglobin | 0.5 mg/mL |
| Goat hemoglobin | 0.5 mg/mL |
| Horse hemoglobin | 20 mg/mL |
| Rabbit hemoglobin | 0.06 mg/mL |

## REFERENCES

1. Simon J.B. Occult Blood Screening for Colorectal Carcinoma: A Critical Review, Gastroenterology, Vol. 1985;88:820.
2. Blebea J. and Ncpherson RA. False-Positive Guaiac Testing With Iodine, Arch Pathol Lab Med, 1985;109:437-40.

## INDEX OF SYMBOLS

| | | | | | |
|---|---|---|---|---|---|
| | Consult instructions for use | | Tests per kit | EC REP | Authorized Representative |
| IVD | For *in vitro* diagnostic use only | | Use by | | Do not reuse |
| 30°C 2°C | Store between 2~30°C | LOT | Lot Number | REF | Catalog# |

Zhejiang Orient Gene Biotech Co.,Ltd
Address: 3787#, East Yangguang Avenue, Dipu Street,
Anji 313300, Huzhou, Zhejiang, China
Tel: +86-572-5226111 Fax: +86-572-5226222
Website: www.orientgene.com

EC REP Shanghai International Holding Corp. GmbH (Europe)
Add: Eiffestrasse 80, 20537 Hamburg, Germany

REF GEFOB-602b

Revision Date: 2023-04-18
B21056-04

# HBV Hepatitis B Virus Combo Rapid Test Cassette (Whole Blood/Serum/Plasma)

For professional *in vitro* diagnostic use only.

## INTENDED USE

HBV Hepatitis B Virus Combo Rapid Test Cassette (Whole Blood/Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B Surface Antigen (HBsAg), Hepatitis B Surface Antibody (HBsAb), Hepatitis B Envelope Antigen (HBeAg), Hepatitis B Envelope Antibody (HBeAb), and Hepatitis B Core Antibody (HBcAb) in human whole blood, serum and plasma.

## SUMMARY

Chronic hepatitis B is a serious, debilitating illness that can cause cirrhosis of the liver, liver cancer and death.
Chronic hepatitis B is the main cause of liver cancer and the tenth leading cause of death worldwide, with 400,000,000 people infected with the virus. Every year, one million people worldwide are expected to die from this infection.
Most people fight off the infection themselves, but approximately 5-10 percent of those infected with the virus become carriers, and an additional 5-10 percent of those infected each year will progress to chronic liver disease, cirrhosis and possibly liver cancer.
HBV Hepatitis B Virus Combo Rapid Test Cassette (Whole Blood/Serum/Plasma) is a rapid test to qualitatively detect the presence of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in human whole blood, serum and plasma without the use of an instrument.[1]

## PRINCIPLE

**HBsAg and HBeAg**

The HBsAg and HBeAg tests are qualitative, two-site sandwich immunoassays for the detection of HBsAg or HBeAg in human whole blood,serum or plasma. The membrane is pre-coated with anti-HBsAg or anti-HBeAg antibodies on the test line region of the strip. During testing, the whole blood, serum or plasma specimen reacts with the particle coated with anti-HBsAg or anti-HBeAg antibodies. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg or anti-HBeAg antibodies on the membrane and generate a colored line. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result.

**HBsAb**

Hepatitis B surface Antibody (HBsAb) is also known as anti-Hepatitis B surface Antigen (anti-HBs). This test is a qualitative, lateral flow immunoassay for the detection of HBsAb in human whole blood, serum or plasma. The membrane is pre-coated with HBsAg on the test line region of the strip. During testing, the whole blood, serum or plasma specimen reacts with the particle coated with HBsAg. The mixture migrates upward on the membrane chromatographically by capillary action to react with HBsAg on the membrane and generate a colored line. The presence of this colored line in the test line region indicates a positive result, while its absence indicates a negative result.

**HBeAb and HBcAb**

Hepatitis B envelope Antibody (HBeAb) is also known as anti-Hepatitis B envelope Antigen (anti-HBe). Hepatitis B core Antibody (HBcAb) is also known as anti-Hepatitis B core Antigen (anti-HBc). These tests are immunoassays based on the principle of competitive binding. During testing, the mixture migrates upward on the membrane chromatographically by capillary action. The membrane is pre-coated with HBeAg or HBcAg on the test line region of the strip. During testing, anti-HBe antibody or anti-HBc antibody, if present in the specimen, will compete with particle coated anti-HBe antibody or anti-HBc antibody for limited amount of HBeAg or HBcAg on the membrane, and no line will form in the test line region, indicating a positive result. A visible colored line will form in the test line region if there is no anti-HBe antibody or anti-HBc antibody in the specimen because all the antibody coated particles will be captured by the antigen coated in the test line region. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

## PRECAUTIONS

- For professional *in vitro* diagnostic use only. Do not use after expiration date.
- Do not eat, drink or smoke in the area where the specimens or kits are handled.
- Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout testing and follow the standard procedures for proper disposal of specimens.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
- Humidity and temperature can adversely affect results.

## STORAGE AND STABILITY

Store as packaged in the sealed pouch either at room temperature or refrigerated (2-30°C). The test cassette is stable through the expiration date printed on the sealed pouch. The test cassette must remain in the sealed pouch until use. DO NOT FREEZE. Do not use beyond the expiration date.

## SPECIMEN COLLECTION AND PREPARATION

- HBV Hepatitis B Virus Combo Rapid Test Cassette (Whole Blood/Serum/Plasma) can be performed using human whole blood, serum and plasma.
- Separate the serum or plasma from blood as soon as possible to avoid hemolysis. Only clear, nonhemolyzed specimens can be used.
- Testing should be performed immediately after the specimens have been collected. Do not leave the specimens at room temperature for prolonged periods. Specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations for the transportation of etiologic agents.

## MATERIALS

**Materials Provided**

• Test cassette  Buffer  Dropper  Package insert

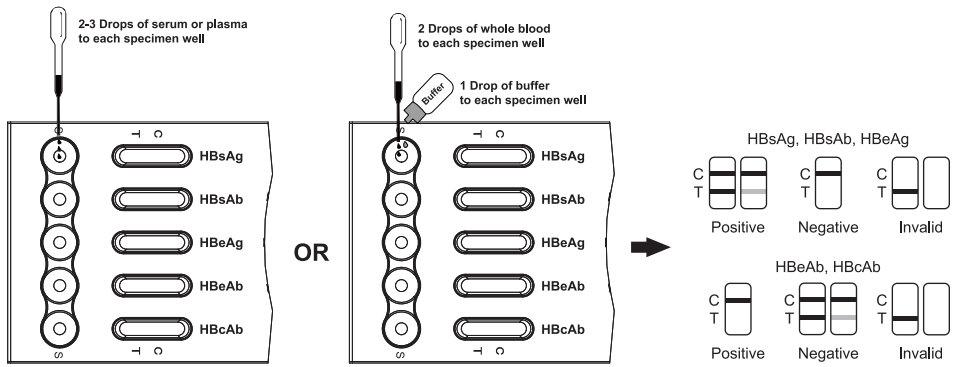
**Materials Required but Not Provided**

• Specimen collection container  Centrifuge (for plasma only)  Timer

## DIRECTIONA FOR USE

**Allow test cassette, specimen, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.**

1. Bring the pouch to room temperature before opening it. Remove the test cassette from the sealed pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
2. Place the test cassette on a clean and level surface.
   **For Serum or Plasma specimens**: Hold the dropper vertically and transfer 2-3 drops of serum or plasma to each specimen well (S) of the test cassette respectively, then start the timer. Avoid trapping air bubbles in the specimen well (S). See illustration below.
   **For Venipuncture Whole Blood specimens**: Hold the dropper vertically and transfer 2 drops of whole blood to the specimen well (S) of the test cassette, then add 1 drop of buffer and start the timer. See illustration below.
3. Wait for the red line(s) to appear. The results should be read at 15 minutes. Do not interpret the results after 20 minutes.




## INTERPRETATION OF RESULTS

**(Please refer to the illustration above)**

WARNING: Do not interpret all 5 tests with the same criteria. Carefully follow the directions below.

**HBsAg, HBsAb, HBeAg**

POSITIVE: Two red lines appear. One line should be in the control line region (C) and another line should be in the test line region (T).
NEGATIVE: One red line appears in the control line region (C). No apparent red or pink line appears in the test line region (T).
INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

**HBeAb, HBcAb**

POSITIVE: One red line appears in the control line region(C), No apparent red or pink line appears in the test region (T).

NEGATIVE: Two red lines appear. One line should be in the control line region (C) and another line should be in the test line region (T).
INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test device. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

## QUALITY CONTROL

A procedural control is included in the test. A red line appearing in the control line region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique.
Control standards are not supplied with this kit; however, it is recommended that positive and negative controls be tested as good laboratory practice to confirm the test procedure and to verify proper test performance.

## LIMITATION

1. The HBV Hepatitis B Virus Combo Rapid Test Cassette (Whole Blood/Serum/Plasma) is for in vitro diagnostic use only. This test should be used for the detection of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in human whole blood, serum or plasma. Neither the quantitative value nor the rate of increase in the concentration of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb can be determined by this qualitative test.
2. The HBV Hepatitis B Virus Combo Rapid Test Cassette (Whole Blood/Serum/Plasma) will only indicate the presence of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in the specimen and should not be used as the sole criteria for the diagnosis of Hepatitis B viral infection.
3. As with all diagnostic tests, all results must be considered with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional follow-up testing using other clinical methods is suggested. A negative result at any time does not preclude the possibility of Hepatitis B Virus infection.

## PERFORMANCE CHARACTERISTICS

**Sensitivity and Specificity**

The HBV Hepatitis B Virus Combo Rapid Test Cassette (Whole Blood/Serum/Plasma) was compared with leading commercial ELISA of HBsAg, HBsAb, HBeAg, HBeAb, HBcAb tests, the results show that the HBV Hepatitis B Virus Combo Rapid Test Cassette (Whole Blood/Serum/Plasma) has a high sensitivity and Specificity.

**HBsAg**

| Method | | ELISA | | Total results |
|---|---|---|---|---|
| HBsAg test cassette | Results | Positive | Negative | |
| | Positive | 145 | 5 | 150 |
| | Negative | 0 | 150 | 150 |
| Total results | | 145 | 155 | 300 |
| Analysis of the results | | | | |
| Relative Sensitivity | | >99.0% | | |
| Relative Specificity | | 96.8% | | |
| Accuracy | | 98.3% | | |

**HBsAb**

| Method | | ELISA | | Total results |
|---|---|---|---|---|
| HBsAb test cassette | Results | Positive | Negative | |
| | Positive | 220 | 2 | 222 |
| | Negative | 0 | 150 | 150 |
| Total results | | 220 | 152 | 372 |
| Analysis of the results | | | | |
| Relative Sensitivity | | >99.0% | | |
| Relative Specificity | | 98.7% | | |
| Accuracy | | 99.5% | | |

**HBeAg**

| Method | | ELISA | | Total results |
|---|---|---|---|---|
| HBeAg test cassette | Results | Positive | Negative | |
| | Positive | 111 | 6 | 117 |
| | Negative | 2 | 332 | 334 |
| Total results | | 113 | 338 | 451 |
| Analysis of the results | | | | |
| Relative Sensitivity | | 98.2% | | |
| Relative Specificity | | 98.2% | | |
| Accuracy | | 98.2% | | |

**HBeAb**

| Method | | ELISA | | Total results |
|---|---|---|---|---|
| HBeAb test cassette | Results | Positive | Negative | |
| | Positive | 103 | 9 | 112 |
| | Negative | 6 | 321 | 327 |
| Total results | | 109 | 330 | 439 |
| Analysis of the results | | | | |
| Relative Sensitivity | | 94.5% | | |
| Relative Specificity | | 97.3% | | |
| Accuracy | | 96.6% | | |

**HBcAb**

| Method | | ELISA | | Total results |
|---|---|---|---|---|
| HBcAb test cassette | Results | Positive | Negative | |
| | Positive | 443 | 4 | 447 |
| | Negative | 17 | 120 | 137 |
| Total results | | 460 | 124 | 584 |
| Analysis of the results | | | | |
| Relative Sensitivity | | 96.3% | | |
| Relative Specificity | | 96.8% | | |
| Accuracy | | 96.4% | | |

**Precision**

**Intra-Assay**

Within-run precision has been determined by using 15 replicates of three specimens containing negative, low positive and high positive of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb. The negative and positive values were correctly identified 99% of the time.

**Inter-Assay**

Between-run precision has been determined by using the same three specimens of negative, low positive and high positive of HBsAg, HBsAb, HBeAg, HBeAb and HBcAb in 15 independent assays. Three different lots of the HBV One Step Hepatitis B Virus Combo Test Device (Whole blood/Serum/Plasma) have been tested over a 3 months period using negative, low positive and high positive specimens. The specimens were correctly identified 99% of the time.

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2. ter Bog F., ten Kate F.J., Cuypers H.T., Leentvaar-KuipersA., Oosting J., Wertheim-van Dillen P.M., Honkoop P, Rasch M.C., de Man R.A., vab Hattum J., Chamelueau R.A., Reesink H.W., Jones E.A., Relation between laboratory results and histological hepatitis activity in individuals postitve for hepatitis B surface antigen and antibodies to hepatitis B e antigen, Lancet 1998 June 351: 1914-8.



Effective date: 2023-02-08
B21445-02

# D-Dimer Rapid Test Cassette (Whole Blood/Plasma)

## INTENDED USE

The D-Dimer Rapid Test Cassette (Whole Blood/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of D-dimer in human whole blood or plasma. It is intended to be used as a screening test and as an aid in the diagnosis of disseminated intravascular coagulation (DIC), deep vein thrombosis (DVT). Any reactive specimen with the D-Dimer Rapid Test Cassette (Whole Blood/Plasma) must be confirmed with alternative testing method(s) and clinical findings.

## INTRODUCTION

During blood coagulation process, fibrinogen is converted to fibrin by the activation of thrombin. The resulting fibrin monomers polymerise to form a soluble gel of non-cross-linked fibrin. This fibrin gel is then converted to cross-linked fibrin by thrombin activated Factor XIII to form an insoluble fibrin clot. Production of plasmin, the major clot-lysing enzyme, is triggered when a fibrin clot is formed. Although fibrinogen and fibrin are both cleaved by the fibrinolytic enzyme plasmin to yield degradation products, only degradation products from cross-linked fibrin contain D-dimer and are called cross-linked fibrin degradation products. Therefore, fibrin derivatives in human blood or plasma containing D-dimer are a specific marker of fibrinolysis.

The D-Dimer Rapid Test Cassette (Whole Blood/Plasma) is a rapid test that qualitative detects the presence of D-dimer in whole blood or plasma specimens at the sensitivity of 500 ng/mL. The test utilizes a combination of monoclonal antibodies to selectively detect elevated levels of D-dimer in whole blood or plasma. At the level of claimed sensitivity, the D-Dimer Rapid Test Cassette (Whole Blood/Plasma) shows no cross-reactivity interference from the related Troponin I, Troponin T, CK-MB, Myoglobin or others at high physiological levels.

## PRINCIPLE

The D-Dimer Rapid Test Cassette (Whole Blood/Plasma) is immunochromatographic assay including D-Dimer specific monoclonal antibody conjugated to colloidal gold particles, second D-Dimer specific monoclonal antibody on test line and Goat anti-mouse IgG antibody on the control line. When the specimen containing D-Dimer is added to sample pad, it moves to conjugate pad and forms a complex (D-Dimer and antibody-gold conjugate). The complex migrates through a nitrocellulose membrane by capillary action and captured at test line which is second D-Dimer specific monoclonal antibody has been bound. The complex is concentrated at test line and a pink or purple line is showed if the D-Dimer concentration is higher than the clinically established cut-off. Uncaptured gold conjugate continues to flow towards control line which Goat anti-mouse IgG is bound and forms a pink or purple color line, indicating test is working as designed and the result is valid. If the control line does not appear, the test result is not valid.

## PRODUCT CONTENTS

The D-Dimer Rapid Test Cassette (Whole Blood/Plasma) containing Anti-D-dimer particles and Anti-D-dimer coated on the membrane.

## MATERIALS SUPPLIED

Test Cassette 2. Pipette Dropper 3. Desiccant 4. Buffer 5. Package Insert

## MATERIAL REQUIRED BUT NOT PROVIDED

Timer 2. Lancing device for whole blood test

## STORAGE AND STABILITY

The kit can be stored at room temperature or refrigerated (2-30°C). The test Cassette is stable through the expiration date printed on the sealed pouch. The test Cassette must remain in the sealed pouch until use. DO NOT FREEZE. Do not use beyond the expiration date.

## WARNINGS AND PRECAUTIONS

1. For professional in vitro diagnostic use only.
2. Do not use after the expiration date indicated on the package. Do not use the test if the foil pouch is damaged. Do not reuse tests.
3. This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not completely guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled by observing usual safety precautions (e.g., do not ingest or inhale).
4. Read the entire procedure carefully prior to testing.
5. Do not eat, drink or smoke in any area where specimens and kits are handled.
6. Handle all specimens as if they contain infectious agents. Observe established precautions against microbiological hazards throughout the procedure and follow standard procedures for the proper disposal of specimens. Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are assayed.
7. Do not interchange or mix reagents from different lots. Do not mix solution bottle caps.
8. Humidity and temperature can adversely affect results.

## SPECIMEN COLLECTION AND PREPARATION

1. The D-Dimer Rapid Test Cassette (Whole Blood/Plasma) is intended for use with human whole blood or plasma specimens only.
2. Only clear, non-hemolyzed specimens are recommended for use with this test. Whole blood or Plasma should be separated as soon as possible to avoid hemolysis.
3. Perform testing immediately after specimen collection. Do not leave specimens at room temperature for prolonged periods. Plasma specimens may be stored at 2-8°C for up to 3 days. For long term storage, specimens should be kept below -20°C. Whole blood collected by venipuncture should be stored at 2-8°C if the test is to be run within 2 days of collection. Do not freeze whole blood specimens. Whole blood collected by fingerstick should be tested immediately.
4. Containers containing anticoagulants such as EDTA, citrate, or heparin should be used for whole blood storage.
5. Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Avoid repeated freezing and thawing of specimens.
6. If specimens are to be shipped, pack them in compliance with all applicable regulations for transportation of etiological agents.
7. Icteric, lipemic, hemolysed, heat treated and contaminated specimens may cause erroneous results.

## TEST PROCEDURE

**Bring tests, specimens, reagents and/or controls to room temperature (15-30°C) prior to testing.**

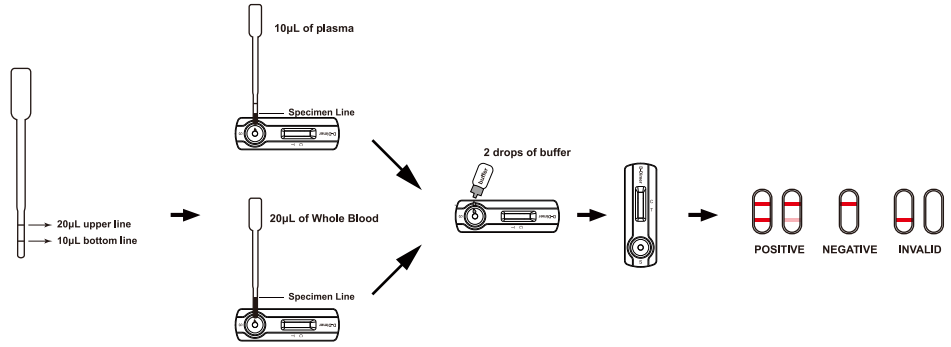
1. Remove the test cassette from the foil pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.
2. Place the test cassette on a clean and level surface.

**For Whole Blood Specimen:** With the 10/20µL mini plastic dropper provided, draw the whole blood specimen to the upper scale line as showed in the following image and then transfer drawn whole blood into the sample well ( S ) of the test device., then add 2 drops of buffer (approximately 80µL) and start the timer. See illustration below.

**For Plasma Specimen:** With the 10/20µL mini plastic dropper provided, draw the plasma specimen to the bottom scale line as showed in the following image and then transfer drawn plasma into the sample well ( S ) of the test device. Then add 2 drops of buffer (approximately 80µL) and start the timer. See illustration below.

*Note: Practice a few times prior to testing if you are not familiar with the mini dropper. For better precision, transfer specimen by pipette capable to deliver 10 and 20µL of volume.*

3. As the test begins to work, color will migrate across the membrane.
4. Wait for the colored band(s) to appear. The result should be read in 10 minutes. Do not interpret the result after 15 minutes.




## INTERPRETATION OF RESULTS

(Please refer to the illustration above)

**Positive:** Two lines appear. One colored line should be in the control line region (C) and another apparent colored line should be in the test line region (T).

**Negative:** One colored line appears in the control line region(C). No line appears in the test line region (T).

**Invalid:** Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test Cassette. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

## QUALITY CONTROL

A procedural control is included in the test. A red line appearing in the control region (C) is the internal procedural control. It confirms sufficient specimen volume and correct procedural technique. Control standards are not supplied with this test. However, it is recommended that positive and negative controls are sourced from a local competent authority and tested as a good laboratory practice, to confirm the test procedure and verify the test performance.

## LIMITATIONS

1. The D-Dimer Rapid Test Cassette (Whole Blood/Plasma) is for in vitro diagnostic use only. This test should be used for the detection of D-dimer in whole blood or plasma specimens only. Neither the quantitative value nor the rate of increase in D-dimer can be determined by this qualitative test.
2. The D-Dimer Rapid Test Cassette (Whole Blood/Plasma) will only indicate the qualitative level of D-dimer in the specimen and should not be used as the sole criteria for the diagnosis of Disseminated Intravascular Coagulopathy (DIC), Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE).
3. During the process of serum is formed, also fibrinogen is converted to fibrin by the activation of thrombin and it also can be detected by D-dimer antibody. So serum specimen can't be used for D-Dimer Rapid Test Device (Whole Blood/Plasma).
4. The D-Dimer Rapid Test Cassette (Whole Blood/Plasma) cannot detect less than 500 ng/mL D-dimer in specimens. A negative result at any time does not preclude the possibility of Disseminated Intravascular Coagulopathy (DIC), Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE).
5. False negative readings can occur if the sample is taken either too early after thrombus formation, if testing is delayed for several days or if the sample was take too later after the occurrence of thromboembolic infarction, because the D-dimer concentration may decrease to normal values after one week already. Additionally, a treatment with anti-coagulants prior sample collection can render the test negative because it prevents thrombus extension.
6. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician. E.g. use "Wells score" for DVT resp. PE, Ultrasound, quantitative laboratory D-Dimer results etc.
7. Some specimens containing unusually high titers of heterophile antibodies or rheumatoid factor (RF) may affect expected results. Even if the test results are positive, further clinical evaluation should be considered with other clinical information available to the physician

## PERFORMANCE CHARACTERISTICS

The D-Dimer Rapid Test Cassette (Whole Blood/Plasma) has been evaluated with a leading commercial D-dimer EIA test using clinical specimens. The results show that the sensitivity of the D-Dimer Rapid Test Cassette (Whole Blood/Plasma) is 98.6% and the specificity is 98.6% relative to the leading EIA test.

| Method | | EIA | | Total Results |
|---|---|---|---|---|
| D-Dimer Rapid Test Cassette | Results | Positive | Negative | |
| | Positive | 71 | 3 | 73 |
| | Negative | 1 | 211 | 212 |
| Total Results | | 72 | 214 | 286 |

Relative Sensitivity: 98.6%
Relative Specificity: 98.6%
Accuracy: 98.6%

## REFERRENCE


1. Gaffney, P.J. D-dimer History of Discovery, Characterisation and Utility of this and other Fibrin Fragments. Fibrinolysis 7 Suppl 2:2-8; 1993
2. Lane, D.A. et al. Characterisation of Serum Fibrinogen and Fibrin Fragments Produced During Disseminated Intravascular Coagulation. Haematology. 40: 609-615; 1978.
3. Scarvelis, D and Wells, P.S. Diagnosis and Treatment of Deep Vein Thrombosis. Can. Med. Assoc. J. 175 (9):1087-92; 2006
4. Bick, R.L. et al. Diagnostic Efficacy of the D-dimer assay in Disseminated Intravascular Coagulation (DIC) Thromb. Res. 65:785-790; 1992.
5. Bick, R.L. et al. Disseminated Intravascular Coagulation: Objective Clinical and Laboratory Diagnosis, Treatment, and Assessment of Therapeutic Response. Semin. Thromb. Hemost. 22(1): 69-88; 1996.
6. Hunt, F.A. et al. Serum Cross-Linked Fibrin (XDP) and Fibrinogen/Fibrin Degradation Products (FDP) in Disorders Associated with Activation of the Coagulation or Fibrinolytic Systems. Br. J. Haematol. 60: 715-722; 1985.
7. Subramanian, R.M. et. al. Does an Immunochromatographic D-dimer exclude acute lower limb deep venous thrombosis? Emer. Med. Austral. 18: 457-463; 2006.
8. Runyon, M.S. et. al. Comparison of the Simplify D-dimer assay performed at the bedside with a laboratory based quantitative D-dimer assay for the diagnosis of pulmonary embolism in a low prevalence emergency department population. Emerg. Med. J. 25:70-75; 2008.

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# DICHIARAZIONE DI CONFORMITA'
*Conformity declaration*

CE

Il sottoscritto, Rinaldo Ruggero legale rappresentante della ditta:
*The undersigned, Rinaldo Ruggero legal representative of the company:*

produttore/*manufacturer*

SYNTESYS S.a.s. di Rinaldo R. & C.

indirizzo/*address*

Via G. Galilei, 10/3 35037 Zona Industriale SELVE DI TEOLO (PADOVA) ITALY

O rappresentante il mandatario autorizzato entro la Unione Europea
*or representing the authorized mandatary within the European Community*

Mandatario autorizzato/*authorized mandatary*

indirizzo/*address*

Dichiara sotto la propria responsabilità che il prodotto/*declares under his own responsability that the product:*

| | |
|---|---|
| Denominazione/*Description* | **Padella per ammalati, urinali uomo e donna, speculum vaginali, tamponcini cotonati, tamponi sterili in provetta, tamponi sterili con terreno Amies e Stuart in provetta/ *Bed pan, Urinal's man and woman, Vaginal speculum, Cotton swab, Sterile swab in test tube, Sterile swab with medium Amies or Stuart in test tube*** |
| Materiale/*Material* | **Polipropilene, Polietilene, Legno/ *Polypropylene, Polyethylene, Wood*** |

È conforme alle disposizioni della direttiva 93/42/CE e smi, concernente i dispositivi medici ed al Decreto Legislativo di recepimento con D.lgs. del 24/02/1997 n° 46/97 e soddisfa a tutti i requisiti specificati.
Il dispositivo è stato classificato appartenente alla classe I° secondo i criteri stabiliti in base a quanto previsto dall'Art. 9 ed allegato IX della direttiva sopra citata /*It meets the EC Directive 93/42 about Medical Device, specifications established by the Italian law n 46/97, dated 24th February 1997. The device was classified as belonging to the 1st class, according to the specifications of the established by the art.9, IX enclosure of the above mentioned directive.*

Dichiara inoltre che la documentazione tecnica di supporto alla presente dichiarazione di conformità è conservata presso gli uffici dell'azienda e sarà posta alla disposizione di chi la richiede/ *declares that all technical documents attached to this conformity statment are filed in our company and can be consulted by any authorized body on demand.*

Data 07.01.2016
Issued on January 7th 2016

SYNTESYS S.A.S.
Il legale rappresentante
Rinaldo Ruggero

SYNTESYS

ISO9001:2008
Cert. N 6574/0

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# DICHIARAZIONE DI CONFORMITA'
## *Conformity declaration*

CE

Il sottoscritto, Rinaldo Ruggero legale rappresentante della ditta:
*The undersigned, Rinaldo Ruggero legal representative of the company:*

produttore/*manufacturer*

SYNTESYS S.a.s. di Rinaldo Ruggero & C.

indirizzo/*address*

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*O rappresentante il mandatario autorizzato entro la Unione Europea* or representing the authorized mandatary within the European Community

Mandatario autorizzato/*authorized mandatary*

indirizzo/*address*

Dichiara sotto la propria responsabilità che il prodotto/*declares under his own responsability that the product:*

| | |
|---|---|
| Denominazione degli articoli prodotti/*Description of Manufacturer* | **Contenitori per urina, contenitori per feci, contenitori universali, Pipette Pasteur, Piastre di Petri, Anse Sterili per batteriologia, Aste a "L", Puntali Eppendorf gialli e blue, cuvette per spettrofotometro, tazzine per campionamento siero, bacchette per distacco ed estrazione del coagulo, pinzette in polistirolo monouso, provette monouso in plastica, tappi alettati per provette diam. 12 mm e 16mm, provette con granuli ed acceleratore, provette sottovuoto per prelievo, Sistema SEDIPLAST, Microprovette, Portavetrini, Vetrini precolorati, Portaprovette, supporti per microprovette, bottiglie per raccolta urine.**<br><br>***Urine container, faeces container, universal container, Pasteur pipette, Petri dishes, Sterile loops, Sterile loops open "L", Eppendorf tips yellow and blue, cuvettes for spectrophotometer, samples cups, Rod to detach clot, disposable forceps, Disposable plastic tubes, winged stoppers for tubes diam. 12mm & 16mm, Test tube with granules and clot activator, vacuum test tube, SEDIPLAST system, micro test tubes, Slides Mailer,"TESTSIMPLETS" slides rack for test tubes, rack for micro test tubes, Bottles for urine collection.*** |

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| | |
|---|---|
| Materiale/*Material* | **Polipropilene, Polistirolo, Polietilene e Polimetilmetacrilato**<br><br>***Polypropylene, Polystyrene, Polyethylene and Polymetilmetacrylate*** |

È conforme alle disposizioni della direttiva 98/79/CE concernente i dispositivi medici diagnostici in vitro e recepito in Italia con D.L. del 08/09/2000 n° 332 allegato 1 (requisiti essenziali) ed è fabbricato in accordo ai requisiti di cui all'Allegato III della sopra citata direttiva / *It meets the CE Directive 98/79 CE about in vitro diagnotic device specifications established by the Italian law n. 332, dated 8th September 2000. The device is made according to the specifications of the III attached of the above-mentioned directive.*

Dichiara inoltre che la documentazione tecnica di supporto alla presente dichiarazione di conformità è conservata presso gli uffici dell'azienda e sarà posta alla disposizione di chi la richiede/*declares that all technical documents attached to this conformity statment are filed in our company and can be consulted by any authorized body on demand.*

Data 07/01/2016
Issued on January 7th 2016

SYNTESYS S.a.s.
Il legale rappresentante
Rinaldo Ruggero

# DICHIARAZIONE DI CONFORMITA' UE
# *EU DECLARATION OF CONFORMITY*

conforme all'Allegato IV del Regolamento (UE) 2017/746 "Dispositivi medico-diagnostici in vitro"
*according to Annex IV of the Regulation (EU) 2017/746 "In vitro diagnostic medical devices"*


fabbricante / *manufacturer*: **ROLL S.R.L.**
**articoli per laboratori analisi - disposable labware**

N° registrazione unico SRN: **IT-MF-000021270**

indirizzo / *address*: **Via Leonardo da Vinci, 24/A**
**35028 PIOVE DI SACCO (PD) - ITALIA**

telefono / *phone*: **+39-0499719511**
fax / *fax*: **+39-0499719543**
posta elettronica / *e-mail*: **roll@tecnomeus.it**


*Identificazione dei prodotti*: **PROVETTE PST 16X100 MM 10 ML CONICHE CON BORDO**

*Product identification*: ***PS CONICAL TEST TUBES 16X100 MM 10 ML WITH RIM***

*Destinazione d'uso* / *Intended use*: ***CAMPIONAMENTO DI LIQUIDI BIOLOGICI***
***SAMPLING OF BIOLOGIC LIQUIDS***

**BASIC UDI-DI**: **805938689TTUBEVZ**

**CND**: **W050301020102**

numero di catalogo / *part number*: **18304**
numero di lotto / *batch number*: **32641**
scadenza / *expiry date*: **31/05/2028**

classificazione dei prodotti / *product identification*: **dispositivi non sterili rientranti nella classe A del regolamento 2017/746, conforme alla regola 5**
***non sterile devices included in the class A regulation (EU) 2017/746, according to rule 5***

**Si dichiara**

sotto la propria esclusiva responsabilità che tutti i dispositivi sopraelencati rispettano le disposizioni applicabili dal regolamento 2017/746 Dispositivi Medico-Diagnostici In Vitro.

La documentazione tecnica richiesta dal succitato regolamento e quella comprovante il rispetto dei Requisiti generali di sicurezza e prestazione di cui all'Allegato I del Regolamento, sono conservati a cura del Fabbricante

***Hereby we declare***

*Under our sole responsability that the above mentioned devices meet the applicable provisions of the Regulation (EU) 2017/746 on "In vitro diagnostic medical devices"*

*The technical documentation, as required by Regulation (EU) 2017/746 and documents in order to prove conformity to general safety and performance requirements as listed in Annex I, are retained under the premises of the Manufacturer*

luogo e data / *place and date*: **PIOVE DI SACCO, 01/07/2023**

firma / *signature*:
**ROLL S.R.L.**
**Quality Assurance**
**Giovanni Chiarin**

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Cert. N.7111/2 Cert. N.6574/2

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**DICHIARAZIONE DI CONFORMITA'**
***Conformity declaration***

CE

**Il sottoscritto, Rinaldo Ruggero legale rappresentante della ditta:**
***The undersigned, Rinaldo Ruggero legal representative of the company:***

produttore/*manufacturer*

**SYNTESYS S.r.l.**

indirizzo/*address*

**Via G. Galilei, 10/3 35037 Zona Industriale SELVE DI TEOLO (PADOVA) ITALY**

**O rappresentante il mandatario autorizzato entro la Unione Europea**
***or representing the authorized mandatary within the European Community***

Mandatario autorizzato/*authorized mandatary*

indirizzo/*address*

Dichiara sotto la propria responsabilità che il prodotto/*declares under his own responsability that the product:*

| | | |
|---|---|---|
| Denominazione/*Description* | **Microprovette tipo Eppendorf in polipr. coniche graduate 1,5 ml c/tappo /***Polypropylene microtubes Eppendorf type conical graduated with cap vol. 1,5 ml* | |
| Lotto/*Lot* | **21184378** | Data di scadenza/expiry date **06.2026** |
| Codice/*Code* | **318766** | |
| Materiale/*Material* | **Polipropilene/** *Polypropylene* | |
| Confezione/*Pack* | **10.000 pezzi/***10.000 pcs.* | |

È conforme alle disposizioni della direttiva 98/79/CE concernente i dispositivi medici diagnostici in vitro e recepito in Italia con D.L. del 08/09/2000 n° 332 allegato 1 (requisiti essenziali) ed è fabbricato in accordo ai requisiti di cui all'Allegato III della sopra citata direttiva / *It meets the CE Directive 98/79 CE about in vitro diagnostic device specifications established by the Italian law n. 332, dated 8th September 2000. The device is made according to the specifications of the III attached of the above-mentioned directive.*

Dichiara inoltre che la documentazione tecnica di supporto alla presente dichiarazione di conformità è conservata presso gli uffici dell'azienda e sarà posta alla disposizione di chi la richiede/ *declares that all technical documents attached to this conformity statement are filed in our company and can be consulted by any authorized body on demand.*

Data 09.09.2021

SYNTESYS S.R.L.
UNIPERSONALE
Il Legale Rappresentante
Rinaldo Ruggero

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Capitale Sociale € 309.600,00
R.E.A. MI 882798
Reg. Imp. C.F. e P.I. MI 01794050151

# CONFORMITY DECLARATION
## Serological Pipettes

References: Invoice FV-22-02443 of OCT. 10, 2022

| Product Code | Description | Lot n. |
|---|---|---|
| 160110 | PS STERILE 1 ML PIPETTE, SINGLE WRAP. | B0890BAA |
| | | **Expiry Date** |
| | | 2027-07 |

**LP ITALIANA declares that all quality qualifications of the products have been respected and that all information on these documents are correct.**

Products are manufactured in accordance with quality system ISO 13485 and ISO 9001 ☑

Products passed visual and functional controls and are in accordance with our internal procedures. ☑

Irradiating process is validated and in accordance with ISO 11137 ☑

Products are irradiated by ionizing radiations at nominal dose of 18 kGy. Batch number 3172253 ☑

**LP ITALIANA SPA**
**Massimiliano Capitanio**
*Quality Assurance Manager*

(This certificate is electronically generated. Signature and check marks are in digital form)

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Cert. N.7111/3 Cert. N.6574/3

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# DICHIARAZIONE DI CONFORMITA' UE
***EU Declaration of conformity***

CE

**Il sottoscritto, Rinaldo Ruggero legale rappresentante della ditta:**
***The undersigned, Rinaldo Ruggero legal representative of the company:***

fabbricante/*manufacturer*

**SYNTESYS S.r.l.**

indirizzo/*address*

**Via G. Galilei, 10/3 35037 Zona Industriale SELVE DI TEOLO (PADOVA) ITALY**

**O rappresentante il mandatario autorizzato entro la Unione Europea**
***or representing the authorized mandatary within the European Community***

Mandatario autorizzato/*authorized mandatary*

indirizzo/*address*

Dichiara sotto la propria responsabilità che il prodotto/*declares under his own responsability that the product:*

| | | | |
|---|---|---|---|
| Denominazione/*Description* | **Cont. urina 120 ml 55x70 mm polipropilene trasparente con tappo a vite bianco inserito con etichetta** / *Transparent polypropylene urine container 120 ml with white screw cap and yellow label* | | |
| Codice/*Code* | **331162** | | |
| Lotto/Lot | **22349397** | Data di scadenza/Expiry date | **11.2027** |
| Classe di rischio / Risk class | **Classe A** / *Class A* | | |
| Numero di registrazione unico (SRN) / Unique registration number (SRN) | **Non disponibile** / *Not available* | | |
| UDI-DI di base / Basic UDI-DI | **805414149CONTURINANZ** | | |

È conforme secondo il Regolamento (UE) 2017/746 concernente i Dispositivi Medico-Diagnostici in vitro e soddisfa tutti i requisiti specificati. Il dispositivo è stato classificato appartenente alla Classe A secondo la Regola 5 dell' Allegato VIII /
It complies with the Regulation (EU) 2017/746 concerning In Vitro Diagnostic Medical Devices and meets all the specified requirements. The device has been classified as belonging to Class A according to Rule 5 of Annex VIII.

Dichiara inoltre che la documentazione tecnica di supporto alla presente dichiarazione di conformità è conservata presso gli uffici dell'azienda e sarà messa a disposizione delle autorità competenti secondo quanto prescritto dall'Art. 10 punto 7 del Regolamento. / *It also declares that the technical documentation supporting this declaration of conformity is kept at the company offices and will be made available to the competent authorities in accordance with the provisions of Art. 10 point 7 of the Regulations.*

Teolo (PD), 17.12.2022

SYNTESYS S.R.L.
UNIPERSONALE
Il Legale Rappresentante
Rinaldo Ruggero

SYNTESYS

Cert. N.7111/2 Cert. N.6574/2

SYNTESYS S.R.L. UNIPERSONALE
VIA G. GALILEI, 10/3 - 35037 Z.I. SELVE DI TEOLO (PD)
TEL. +39 049 9903866 R.A. FAX +39 049 9903867
C.F./P.I./N.REG.IMP. PADOVA 03573950288
REA PD-320123 - CAP.SOC. 20.700,00€
E-MAIL INFO@SYNTESYS.IT - WEB WWW.SYNTESYS.IT
PEC POSTA@PEC.SYNTESYS.IT

# AUTHORIZATION LETTER

We, **Syntesys S.R.L.** having a registered office at Via G. Galilei 10/3, 35037 Selve di Teolo - PD - Italy, assign **Sanmedico SRL** having a registered office at A.Corobceanu str., apt. 9, Chişinău MD-2012, Moldova, as authorized representative.

We declare that the company mentioned above is authorized to register, notify, renew or modify the registration of medical devices on the territory of the Republic of Moldova.

This letter is valid till 28.08.2024

Teolo, 28.08.2023

SYNTESYS S.R.L.
UNIPERSONALE
Via G. Galilei, 10/3 - 35037 Z.I. Selve - Teolo (PD)
C.F./P.I./R.I. PD: 03573950288 - Cap. Soc. 20.700,00 €
Tel. 049 9903866 - Fax 049 9903867

Rinaldo Ruggero
CEO and Legal Representative
SYNTESYS S.R.L.

IQNET

Building trust together.

# Certificate

**CISQ/ICIM S.P.A.** has issued an IQNet recognized certificate that the organization:

## SYNTESYS S.R.L.

**Head Office and Operative Unit**

**Via G. Galilei, 10/1-2-3 - Zona Industriale - I-35037 Selve di Teolo (PD)**

**Operative Units**

**Via G. Galilei, 16/1 - Zona Industriale - I-35037 Selve di Teolo (PD)**

**Via San Benedetto, 48/A - Zona Industriale - I-35037 Selve di Teolo (PD)**
**Via G. Galilei, 3 - Zona Industriale - I-35037 Selve di Teolo (PD)**

has implemented and maintains a/an

**Quality Management System**

for the following scope:

**Trading of products for laboratory analysis. Manufacturing of products for laboratory analysis and sanitary products. Design and production management of sterile swabs for the collection and the preservation of biological samples, also for surgical application, with or without transport medium.**

which fulfils the requirements of the following standard:

**ISO 9001:2015**

| | |
|---|---|
| Issued on: | **2022-06-05** |
| First issued on: | **2013-06-05** |
| Expires on: | **2025-06-04** |

This attestation is directly linked to the IQNet Partner's original certificate and shall not be used as a stand-alone document.

Registration Number: **IT-83562**

**Alex Stoichitoiu**
President of IQNET

**Mario Romersi**
President of CISQ

CISQ

This attestation is directly linked to the IQNET Member's original certificate and shall not be used as a stand-alone document.

**IQNET Members*:**
**AENOR** Spain **AFNOR Certification** France **APCER** Portugal **CCC** Cyprus **CISQ** Italy **CQC** China **CQM** China **CQS** Czech Republic **Cro Cert** Croatia **DQS Holding GmbH** Germany **EAGLE Certification Group** USA **FCAV** Brazil **FONDONORMA** Venezuela **ICONTEC** Colombia **ICS** Bosnia and Herzegovina **Inspecta Sertifiointi Oy** Finland **INTECO** Costa Rica **IRAM** Argentina **JQA** Japan **KFQ** Korea **LSQA** Uruguay **MIRTEC** Greece **MSZT** Hungary **Nemko AS** Norway **NSAI** Ireland **NYCE-SIGE** México **PCBC** Poland **Quality Austria** Austria **SII** Israel **SIQ** Slovenia **SIRIM QAS International** Malaysia **SQS** Switzerland **SRAC** Romania **TSE** Turkey **YUQS** Serbia

* The list of IQNET Members is valid at the time of issue of this certificate. Updated information is available under www.iqnet-certification.com

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CISQ is a member of

IQNET

The International Certification Network

www.iqnet-certification.com

ICIM

CERTIFICATO N.
*CERTIFICATE No.* **6574/3**

SI CERTIFICA CHE IL SISTEMA DI GESTIONE PER LA QUALITÀ DI
*WE HEREBY CERTIFY THAT THE QUALITY MANAGEMENT SYSTEM OPERATED BY*

# SYNTESYS S.R.L.

**Sede e Unità Operativa**

Via G. Galilei, 10/1-2-3 - Zona Industriale - 35037 Selve di Teolo (PD) – Italia

*Commercializzazione di prodotti per analisi di laboratorio. Produzione di prodotti per analisi di laboratorio e articoli sanitari. Progettazione e gestione della produzione di tamponi sterili per la raccolta e la conservazione di campioni biologici, anche in ambito chirurgico, con o senza terreno di trasporto.*

**Unità Operative**

Via G. Galilei, 16/1 - Zona Industriale - 35037 Selve di Teolo (PD) – Italia *
Via San Benedetto, 48/A - Zona Industriale - 35037 Selve di Teolo (PD) – Italia *
Via G. Galilei, 3 - Zona Industriale - 35037 Selve di Teolo (PD) – Italia *
** Magazzino.*

È CONFORME ALLA NORMA / *IS IN COMPLIANCE WITH THE STANDARD*

## UNI EN ISO 9001:2015

Sistema di Gestione per la Qualità / *Quality Management System*

PER LE SEGUENTI ATTIVITÀ / *FOR THE FOLLOWING ACTIVITIES*

**EA: 29 - 14**

Commercializzazione di prodotti per analisi di laboratorio. Produzione di prodotti per analisi di laboratorio e articoli sanitari. Progettazione e gestione della produzione di tamponi sterili per la raccolta e la conservazione di campioni biologici, anche in ambito chirurgico, con o senza terreno di trasporto.

*Trading of products for laboratory analysis. Manufacturing of products for laboratory analysis and sanitary products. Design and production management of sterile swabs for the collection and the preservation of biological samples, also for surgical application, with or without transport medium.*

Riferirsi alla documentazione del Sistema di Gestione per la Qualità aziendale per l'applicabilità dei requisiti della norma di riferimento.
*Refer to the documentation of the Quality Management System for details of application to reference standard requirements.*

Il presente certificato è soggetto al rispetto del documento ICIM "Regolamento per la certificazione dei sistemi di gestione" e al relativo Schema specifico.
*The use and the validity of this certificate shall satisfy the requirements of the ICIM document "Rules for the certification of company management systems" and specific Scheme.*

Per informazioni puntuali e aggiornate circa eventuali variazioni intervenute nello stato della certificazione di cui al presente certificato, si prega di contattare il n° telefonico +39 02 725341 o indirizzo e-mail info@icim.it.
*For timely and updated information about any changes in the certification status referred to in this certificate, please contact the number +39 02 725341 or email address info@icim.it.*

| DATA EMISSIONE | EMISSIONE CORRENTE | DATA DI SCADENZA |
|---|---|---|
| *FIRST ISSUE* | *CURRENT ISSUE* | *EXPIRING DATE* |
| 05/06/2013 | 05/06/2022 | 04/06/2025 |

*Vincenzo Delacqua*
Rappresentante Direzione / Management Representative
**ICIM S.p.A.**
Piazza Don Enrico Mapelli, 75 – 20099 Sesto San Giovanni (MI)
www.icim.it

ACCREDIA
L'ENTE ITALIANO DI ACCREDITAMENTO

SGQ N° 004 A

CISQ

www.cisq.com

CISQ è la Federazione Italiana di Organismi di Certificazione dei sistemi di gestione aziendale. CISQ is the Italian Federation of management system Certification Bodies.

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# Certificate

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## SYNTESYS S.R.L.

**Head Office and Operative Unit**

**Via G. Galilei, 10/1-2-3 - Zona Industriale - I-35037 Selve di Teolo (PD)**

**Operative Units**

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which fulfils the requirements of the following standard:

## ISO 13485:2016

| | |
|---|---|
| Issued on: | **2022-06-05** |
| First issued on: | **2014-06-21** |
| Expires on: | **2025-06-04** |

This attestation is directly linked to the IQNet Partner's original certificate and shall not be used as a stand-alone document.

Registration Number: **IT-93779**

**Alex Stoichitoiu**
President of IQNET

**Mario Romersi**
President of CISQ

CISQ