Toxoplasma gondii

IgM μ-capture ELISA

Enzyme immunoassay for the qualitative determination of IgM-class antibodies against Toxoplasma gondii in human serum
Only for in-vitro diagnostic use

Product Number: TOXM0460 (96 determinations)

CONTENTS

1. INTRODUCTION

Toxoplasma gondii is a small intracellular parasite, whose live cycle has a sexual and an asexual phase. Sexual development is restricted to the intestinal cells of (probably exclusively) cats; the oocysts formed are excreted and due to their resistant cell walls they may be infectious under advantageous circumstances for at least 1 year. Animals and man are intermediate hosts for the asexual proliferation of T. gondii: the ingested parasites will proliferate explosively within the host cells lysing them eventually. They disseminate throughout the body via circulation and lymphatic system and though may infect any cell type. In muscle and brain cells cysts are formed which are spheroidal and about 5-100 µm in diameter. Cysts are virtually immortal in the intermediate host.
Toxoplasma gondii is the most common parasite in humans, but its abundance (7-80%) is highly dependent on the geographic area, the socioeconomic status and the nutritional customs. Infection only rarely causes toxoplasmosis and usually clinical symptoms are absent, but may produce severe problems in immunosuppressed persons and fetus.
Because only a primary infection during pregnancy may be dangerous and even fatal for the unborn (the probability of congenital infection is about 50%), the recent onset of an infection must be excluded.
In pregnant women in over 98% of cases, the absence of IgM excludes the possibility of recent infection. In newborns the very presence of anti-toxoplasma IgM is sufficient to confirm a congenital toxoplasmosis, since maternal IgM, unlike IgG, does not cross the placental barrier. But a significant number of infected infants do not develop detectable IgM levels and thus are false negative. In immunosuppressed patients toxoplasmosis causes severe complications mostly by reactivation of an earlier latent infection.

Species	Disease	Symptoms	Mechanism of infection
Toxoplasma gondii	Toxoplasmosis	Aquired Toxoplasmosis: lymphadenopathy, Chorioretintis Congenital Toxoplasmosis: hydrocephalus, Microcephaly, intracranial calcifications, chronical chorioretinitis	Direct: ingestion of oocysts (cats) by food, including water contaminated by feces of cats or contaminated soil. Indirect: Ingestion of cysts by eating raw or insufficiently cooked meat, esp. pork. Congenital infection of the fetus.

Infection may be identified by:
- PCR
- Indirect immunofluorescence (IIF)
- Serology: Detection of antibody production by ELISA

2. INTENDED USE

The NovaTec Toxoplasma gondii IgM µ-capture ELISA is intended for the qualitative determination of IgM class antibodies against Toxoplasma gondii in human serum.

3. PRINCIPLE OF THE ASSAY

The qualitative immunoenzymatic determination of IgM-class antibodies against Toxoplasma gondii is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.
The Toxoplasma IgM ELISA is an IgM-µ-capture ELISA. Toxoplasma antigen is prepared by purification of Toxoplasma gondii propagated in vitro HEp-2 wells. Peroxidase (PO) is then covalently conjugated to this antigen. For the reduction of non-specific reactivity, unlabeled control antigen (i.e. Hep-2 cellular components) is added to this labeled antigen.
Microtiter strip wells are precoated with anti-human IgM-class antibodies to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase labelled Toxoplasma gondii antigen conjugate is added. This conjugate binds to the captured Toxoplasma-specific antibodies. The immune complex formed by the bound conjugate is visualized with Tetramethylbenzidine (TMB) substrate which gives a blue reaction product.
The intensity of this product is proportional to the amount of Toxoplasma gondii-specific IgM antibody in the specimen. Sulfuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance at 450 nm is read using an ELISA microwell plate reader.

4. MATERIALS

4.1. Reagents supplied

- **Microtiterplate (IgM):** 12 breakapart 8-well snap-off strips coated with anti-human IgM-class antibodies; vacuum sealed, in resealable aluminium foil.
- **IgM Sample Diluent:** 1 bottle containing 110 ml of ready to use buffer for sample dilution; pH 7.2 ± 0.2; colored blue.
- **Stop Solution:** 1 bottle containing 15 ml. Ready to use sulfuric acid, 0.2 mol/l; red cap.
- **Washing Solution (10x conc.):** 1 bottle containing 60 ml of a 10-fold concentrated buffer for washing the wells; pH 7.2 ± 0.2.
- **T. gondii Antigen Conjugate (100x conc.):** 1 vial containing 0.25 ml of peroxidase labelled Toxoplasma-specific antigen; blue cap.
- **T. gondii Conjugate Additive (100x conc.):** 1 vial containing 0.25ml of unlabeled Toxoplasma-specific antigen; black cap.
- **T. gondii Antigen Conjugate Diluent:** 1 bottle containing 20ml of a ready to use buffer for conjugate dilution; pH 7.2 ± 0.2; colored blue, blue cap.
- **TMB Substrate (10x conc.):** 1 vial containing 1.5ml 3,3',5,5'-tetramethylbenzidine (TMB); 10-fold concentrated, white cap
- **TMB Diluent:** 1 bottle containing 15ml of a ready to use buffer for TMB Substrate dilution; pH 7.2 ± 0.2, white cap
- **T. gondii IgM Cut Off Serum:** 1 vial containing 1.8ml; colored green; green cap.
- **T. gondii IgM Positive Control:** 1 vial containing 1 ml; colored red; ready to use; red cap.
- **T. gondii IgM Negative Control:** 1 vial containing 1 ml; colored yellow; ready to use; yellow cap.

4.2. Materials supplied

- 1 Strip holder
- 2 Cover foils
- 1 Test protocol
- 1 distribution and identification plan

4.3. Materials and Equipment needed

- ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620nm
- Incubator 37°C
- Manual or automatic equipment for rinsing wells
- Pipettes to deliver volumes between 10 and 1000 µl
- Vortex tube mixer
- Deionised or (freshly) distilled water
- Disposable tubes
- Timer

5. STABILITY AND STORAGE

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

6. REAGENT PREPARATION

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

6.1. Coated Snap-off Strips

The ready to use breakapart snap-off strips are coated with anti-human IgM-class antibodies. Store at 2...8°C. The strips are vacuum sealed. *Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the dessiccant supplied and stored at 2...8 °C; stability until expiry date.*

6.2. Toxoplasma gondii Antigen Conjugate

For the preparation of the T. gondii Antigen Conjugate use the T. gondii Antigen Conjugate, the T. gondii Conjugate Additive and the T. gondii Antigen Conjugate Diluent.

The vial labeled **T. gondii Antigen Conjugate** contains 0.25ml of a 100-fold concentrated solution with Toxoplasma-specific antigen, horseradish peroxidase, buffer, stabilizers and preservatives. Dilute as discribed below. The solution has to be stored at 2...8°C.

The vial labeled **T. gondii Conjugate Additive** contains 0.25ml of a 100-fold concentrated solution with Toxoplasma-specific antigen, buffer, stabilizers and preservatives. Dilute as discribed below. The solution has to be stored at 2...8°C.

The bottle labeled **T. gondii Antigen Conjugate Diluent** contains 20ml of a buffer, stabilizers, preservatives and an inert blue dye. It is used for the dilution of the POD and the Control Antigen. This ready to use solution has to be stored at 2...8°C.

For the preparation of the T. gondii Antigen Conjugate dilute the T. gondii POD Antigen and the T. gondii Control Antigen 1 + 100 with the T. gondii Antigen Conjugate Diluent.
For example: Mix per 8-well strip 1ml T. gondii Antigen Conjugate Diluent with 10µl T. gondii Antigen Conjugate and 10µl T. gondii Conjugate Additive.
After first opening stability until expiry date when stored at 2…8°C.

6.3. Controls

The vials labelled with Positve and Negative Control contain a ready to use control solution. It has to be stored at 2...8°C. *After first opening stability until expiry date when stored at 2…8°C.*

6.4. IgM Sample Diluent

The bottle contains 110ml phosphate buffer, stabilizers, preservatives and an inert blue dye. It is used for the dilution of the patient specimen. This ready to use solution has to be stored at 2...8°C. *After first opening stability until expiry date when stored at 2…8°C.*

6.5. Washing Solution (10xconc.)

The bottle contains 60ml of a concentrated buffer, detergents, stabilizers and preservatives. Dilute washing solution 1:10; e.g. 10 ml washing solution + 90 ml fresh and germ free redistilled water. The diluted buffer will keep for at least four weeks if stored at 2...8°C.
Crystals in the solution disappear by warming up to 37 °C in a water bath.

6.6. TMB Substrate Solution

For the preparation of the TMB Substrate Solution use the TMB Substrate and the TMB Diluent.

The vial labeled **TMB Substrate** contains 1.8ml of a tetramethylbenzidine/hydrogen peroxide system. The solution is 10-fold concentrated and has to be stored at 2...8°C, away from the light. Dilute as discribed below.

The bottle labeled **TMB Diluent** contains 15ml of a buffer, stabilizers and preservatives. The solution is ready to use and has to be stored at 2...8°C.

For the preparation of the TMB Substrate Solution dilute the TMB Substrate 1:10 with TMB Diluent, e.g. dilute per 8-well strip 100µl TMB Substrate with 900µl TMB Diluent.
The solution should be colourless or have a slight blue tinge. If the substrate turns a darker blue, it may have become contaminated and should be discharged.
After first opening stability until expiry date when stored at 2…8°C.

6.7. Stop Solution

The bottle contains 15ml 0.2 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.
After first opening stability until expiry date..

7. SPECIMEN COLLECTION AND PREPARATION

Use human serum samples with this assay. If the assay is performed within 24 hours after sample collection, the specimen should be kept at 2...8°C; otherwise they should be aliquoted and stored deep-frozen (-20 to -70°C). If samples are stored frozen, mix thawed samples well before testing. *Avoid repeated freezing and thawing.*

7.1. Sample Dilution

Before assaying all samples should be diluted 1+100 with IgM Sample Diluent. Dispense 10µl sample and 1ml IgM Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex. *Positive and negative controls are ready to use and must not be diluted.*

8. ASSAY PROCEDURE

8.1. Test Preparation

Please read the test protocol carefully **before** performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Prepare the required volume of Washing Solution, T. gondii Antigen Conjugate and TMB Substrate Solution (see "5. Reagent Preparation").
Please allocate at least:

1 well	(e.g. A1)	for the substrate blank,
2 wells	(e.g. B1+C1)	for the negative control,
2 wells	(e.g. D1+E1)	for the positive control and
3 wells	(e.g. F1+G1+H1)	for the IgM Cut Off Serum

Controls and patient samples should be determined in duplicate.

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

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

Adjust the incubator to 37° ± 1°C.

1. Dispense 100µl controls, IgM cut off Serum and diluted samples into the respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. **Incubate for 1 hour ± 5 min at 37±1°C.**
4. When incubation has been completed, remove the foil, aspirate the content off the wells and wash each well five times with 300µl of washing solution. Avoid overflows from the reaction wells. The soak time between each wash cycle should be >5sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step!

   Note: Washing is critical! Insufficient washing results in poor precision and falsely elevated absorbance values.
5. Dispense 100µl T. gondii Antigen Conjugate into all wells except for the blank well (e.g. A1). Cover with foil.
6. **Incubate for 1 hour ± 5 min at 37±1°C.** *Do not expose to direct sunlight.*
7. Repeat step 4.
8. Dispense 100µl TMB Substrate Solution into all wells
9. **Incubate for exactly 30 min at room temperature (20 to 25°C) in the dark.**
10. Dispense 100µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. *Any blue color developed during the incubation turns into yellow.*

    Note: Highly positive patient samples can cause dark precipitates of the chromogen! These precipitates have an influence when reading the optical density. Predilution of the sample with physiological sodium chloride solution, for example 1+1, is recommended. Then dilute the sample 1+100 with dilution buffer and multiply the results in NTU by 2.
11. Measure the absorbance of the specimen at 450/620nm within 30 min after addition of the Stop Solution.

8.2. Measurement

Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1.

If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!

Measure the absorbance of all wells at 450 nm and record the absorbance values for each control and patient sample in the distribution and identification plan.

Dual wavelength reading using 620 nm as reference wavelength is recommended.

Where applicable calculate the mean absorbance values of all duplicates.

9. RESULTS

9.1. Calculation of Results

Calculate the mean absorbance value of the T. gondii IgM Cut Off Serum, the negative control, the positive control and the patient specimen.
The abundance of Toxoplasma gondii IgM is expressed in NovaTec Units (NTU) and is calculated as follows:

$$\text{T. gondii IgM abundance} = \frac{\text{(mean) absorbance of control or patient specimen}}{\text{mean absorbance of cut off Serum}} = \text{NovaTec Units (NTU)}$$

9.2. Assay Validation Criteria

In order for an assay to be considered valid, the following criteria must be met:

- **Substrate blank** in A1: Absorbance value **lower than 0.100.**
- **Negative control** in B1 and C1: T. gondii IgM abundance **lower than 0.7 NTU.**
- **Positive control** in D1 and E1: T. gondii IgM abundance **higher than 2.0 NTU.**
- **Cut off Serum** in F1, G1 and H1: Absorbance value **between 0.2 and 0.6.**

9.3. Interpretation of Results

Samples are considered **POSITIVE** if the abundance is higher than 1.1 NTU.

Samples with an abundance between 0.9 and 1.1 NTU can not be considered as clearly positive or negative
→ **grey zone**
It is recommended to confirm the results by testing the sample again in duplicate. If results in the second test are again in the grey zone a second serum sample should be tested and judged for a change in result.

Samples are considered **NEGATIVE** if the abundance is lower than 0.9 NTU.

10. LIMITATIONS OF THE PROCEDURE

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data.
In immunocompromized patients and newborns serological data only have restricted value.

11. PRECAUTIONS AND WARNINGS

- Only for in-vitro diagnostic use.
- All components of human origin used for the production of these reagents have been tested for anti-HIV antibodies, anti-HCV antibodies and HBsAg and have been found to be non-reactive. Nevertheless, all materials should still be regarded and handled as potentially infectious.
- Do not interchange reagents or strips of different production lots.
- No reagents of other manufacturers should be used along with reagents of this test kit.
- Do not use reagents after expiry date stated on the label.
- Use only clean pipette tips, dispensers, and lab ware.
- Do not interchange screw caps of reagent vials to avoid cross-contamination.
- Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
- After first opening and subsequent storage check conjugate and control vials for microbial contamination prior to further use.
- To avoid cross-contamination and falsely elevated results pipette patient samples and dispense conjugate without splashing accurately to the bottom of wells.

12. LITERATURE

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13. ORDERING INFORMATION

Prod. No.:	TOXM0460	Toxoplasma gondii IgM µ-capture ELISA (96 Determinations)

SCHEME OF THE ASSAY

Toxoplasma gondii IgM µ-capture ELISA

Assay Preparation

Prepare reagents and samples as described.
Establish the distribution and identification plan for all specimens and controls on the result sheet supplied in the kit.
Select the required number of microtiter strips or wells and insert them into the holder.
Prepare the required volume of Washing Solution, T. gondii Anitgen Conjugate and TMB Substrate Solution

Assay Procedure

	Substrate blank (e.g. A1)	Negative control	Positive control	Cut Off Serum	Sample (diluted 1+100)
Negative control	-	100µl	-	-	-
Positive control	-	-	100µl	-	-
Cut Off Serum	-	-	-	100µl	-
Sample (diluted 1+100)	-	-	-	-	100µl
Cover wells with foil supplied in the kit **Incubate for 1 h at 37°C** Wash each well five times with 300µl of washing solution					
Conjugate	-	100µl	100µl	100µl	100µl
Cover wells with foil supplied in the kit **Incubate for 1 h at 37°C** Wash each well five times with 300µl of washing solution					
TMB Substrate	100µl	100µl	100µl	100µl	100µl
Incubate for 30 min at room temperature in the dark					
Stop Solution	100µl	100µl	100µl	100µl	100µl
Photometric measurement at 450 nm (reference wavelength: 620 nm)					

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