Toxoplasma gondii

IgM µ-capture– ELISA

Enzyme immunoassay for the qualitative determination of IgM-class antibodies against Toxoplasma gondii in human serum or plasma

For laboratory research only.
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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Disease</th>
<th>Symptoms</th>
<th>Mechanism of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxoplasma gondii</td>
<td>Toxoplasmosis</td>
<td>Acquired Toxoplasmosis: lymphadenopathy, Chorioretinitis</td>
<td>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.</td>
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<tr>
<td></td>
<td></td>
<td>Congenital Toxoplasmosis: hydrocephalus, Microcephaly, intracranial calcifications, chronical chorioretinitis</td>
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</table>

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

2. INTENDED USE

The GenWay Toxoplasma gondii IgM µ-capture ELISA is intended for the qualitative determination of IgM class antibodies against Toxoplasma gondii in human serum or plasma (citrate).

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. 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 a complex of antigen and horseradish peroxidase labelled Toxoplasma gondii antibody is added. This complex binds to the captured Toxoplasma specific IgM antibodies. This immune complex 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. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microowell plate reader.

4. MATERIALS

4.1. Reagents supplied
- Microtiterplate (IgM): 12 breakapart 8-well snap-off strips coated with anti-human IgM-class antibodies; in resealable aluminium foil.
- Sample Diluent*: 1 bottle containing 100 ml of ready to use buffer for sample dilution; pH 7.2 ± 0.2; coloured yellow.
- Stop Solution: 1 bottle containing 15 ml ready to use sulphuric acid, 0.2 mol/l; red cap.
- Washing Solution (20x conc.)*: 1 bottle containing 50 ml of a 20-fold concentrated buffer for washing the wells; pH 7.2 ± 0.2, white cap.
- T. gondii Conjugate lyoph.: 1 vial containing lyophilized conjugate comprising of a complex of Toxoplasma antigen-monoclonal antibody anti-P30 conjugated with horseradish peroxidase (HRP).
- T. gondii Conjugate Diluent: 1 bottle containing 14 ml of a ready to use buffer for conjugate dilution containing monoclonal anti-toxoplasma P30 conjugated with HRP and stabilizers; pH 7.2 ± 0.2; coloured red, white cap.
3. TMB Substrate: 15 ml 3,3',5,5'-tetramethylbenzidine (TMB); ready to use, yellow cap.

4. T. gondii IgM Cut Off Control**: 1 vial containing 3.0 ml; coloured yellow; ready to use; green cap.

5. T. gondii IgM Positive Control**: 1 vial containing 2.0 ml; coloured yellow; ready to use; red cap.

6. T. gondii IgM Negative Control**: 1 vial containing 2.0 ml; coloured yellow; ready to use; blue cap.

* contains 0.1% Bronidox L after dilution
** contains 0.1% Kathon

4.2. Materials supplied

- 3 empty, labelled vials (white with white cap) for storage of aliquots of reconstituted conjugate at -20°C
- 1 Strip holder
- 1 Cover foil
- 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. Immediately after removal of strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8°C; stability until expiry date.

6.2. Toxoplasma gondii Conjugate

Reconstitute the lyophilized vial with 5 ml of Conjugate Diluent. It is recommended to add the volume of Conjugate Diluent into the vial, close the cap, wait 15 min at room temperature and gently mix in order to avoid foaming. Transfer the reconstituted solution into the Conjugate Diluent vial. The final volume will be 14 ml. The reconstituted complex is stable for 2 months at +2…+8°C.

After first use it is recommended to aliquot the reconstituted conjugate into the supplied vials (s. 4.2.) and store the aliquots at -20°C. Aliquot size should be adapted to the conjugate volume usually required for one test run in the particular lab. This is to avoid repeated freezing and thawing. In general storage at -20°C is possible in polypropylene (PP) vials like Eppendorf micro test tubes. At -20°C the reconstituted conjugate is stable until the expiry date. Avoid repeated freezing and thawing.

6.3. Controls

The vials labelled with Positive Control, Negative Control and Cut off Control contain a ready to use control solution. They have to be stored at 2…8°C. After first opening stability until expiry date when stored at 2…8°C.

6.4. Sample Diluent

The bottle contains 100 ml phosphate buffer, stabilizers, preservatives and an inert yellow 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 (20xconc.)

The bottle contains 50 ml of a concentrated buffer, detergents, stabilizers and preservatives. Dilute washing solution 1+19; e.g. 10 ml washing solution + 190 ml fresh and germ free redistilled water. The diluted buffer will keep for 5 days if stored at room temperature. Crystals in the solution disappear by warming up to 37 °C in a water bath. After first opening the concentrate is stable until the expiry date.

6.6. TMB Substrate Solution

The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2…8°C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away. After first opening stability until expiry date when stored at 2…8°C.
6.7. Stop Solution
The bottle contains 15 ml 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 or plasma (citrate) samples with this assay. If the assay is performed within 5 days 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 Sample Diluent. Dispense 10µl sample and 1ml Sample Diluent into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

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. The following test procedure is only validated for manual procedure. If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300µl to 350µl to avoid washing effects. 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 and T. gondii Conjugate (see “6. Reagent Preparation”).

Please allocate at least:
1 well (e.g. A1) for the substrate blank,
1 well (e.g. B1) for the negative control,
2 wells (e.g. C1+D1) for the cut-off control and
1 well (e.g. E1) for the positive control.

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 100 µl controls and diluted samples into the respective wells. Leave well A1 for substrate blank.
2. Cover well 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 three times with 300 µl 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 100 µl T. gondii 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 100 µl TMB Substrate Solution into all wells
9. Incubate for exactly 30 min at room temperature (20 to 25°C) in the dark.
10. Dispense 100 µl Stop Solution into all wells in the same order and at the same rate as for the TMB Substrate Solution. Any blue colour 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. Run Validation Criteria
In order for an assay to be considered valid, the following criteria must be met:
- **Substrate blank** in A1: Absorbance value < 0.100.
- **Negative control** in B1: Absorbance value < cut-off
- **Cut-off control** in C1 and D1: Absorbance value 0.150 – 1.30.
- **Positive control** in E1: Absorbance value > cut-off.

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

9.2. Calculation of Results
The cut-off is the mean absorbance value of the Cut-off control determinations.

\[ \text{Cut-off} = \frac{0.39 + \text{absorbance value Cut-off control}}{2} \]

**Example:**
\[ \text{Cut-off} = \frac{0.39 + 0.37}{2} = 0.38 \]

9.3. Interpretation of Results
Samples are considered **POSITIVE** if the absorbance value is higher than 10% over the cut-off.
Samples with an absorbance value of 10% above or below the cut-off should not be considered as clearly positive or negative → **grey zone**

It is recommended to repeat the test again 2 - 4 weeks later with a fresh sample. If results in the second test are again in the grey zone the sample has to be considered **NEGATIVE**.

Samples are considered **NEGATIVE** if the absorbance value is lower than 10% below the cut-off.

9.3.1. Results in GenWay Units
Patient (mean) absorbance value x 10 \[ \text{GenWay-Units} = \frac{\text{NTU}}{0.38} \]

\[ \text{Example:} \quad \frac{1.786 \times 10}{0.38} = 47 \text{ NTU (GenWay Units)} \]

Cut-off: 10 NTU
Grey zone: 9-11 NTU
Negative: <9 NTU
Positive: >11 NTU

10. SPECIFIC PERFORMANCE CHARACTERISTICS

10.1. Precision

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<td>13.25</td>
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<td>high positive Sera</td>
<td>11</td>
<td>48.06</td>
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<table>
<thead>
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<th></th>
<th>n</th>
<th>mean[OD]</th>
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</thead>
<tbody>
<tr>
<td>positive Sera</td>
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</tr>
<tr>
<td>high positive Sera</td>
<td>20</td>
<td>1.725</td>
<td>3.2</td>
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</table>

10.2. Diagnostic Specificity
The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is >95%.

10.3. Diagnostic Sensitivity
The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 95.2%.

10.4. Interferences
Interferences with hemolytic, lipemic or icteric sera are not observed up to a concentration of 10 mg/ml hemoglobin, 5 mg/ml triglycerides and 0.2 mg/ml bilirubin.

**Note:** The results refer to the groups of samples investigated; these are not guaranteed specifications.
11. 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.

To estimate (primary or recurrent) T. gondii infections by serology it is advised to test serum pairs. The second pair can be drawn 14 to 21 days after the first. Each serum pair should be tested at the same day and in the same test to allow interpretation of significant differences in antibody levels. It is advised to perform a combination of IgM, IgA and IgG testing.

12. PRECAUTIONS AND WARNINGS

- In compliance with article 1 paragraph 2b European directive 98/79/EC the use of the in vitro diagnostic medical devices is intended by the manufacturer to secure suitability, performances and safety of the product. Therefore the test procedure, the information, the precautions and warnings in the instructions for use have to be strictly followed. The use of the testkits with analyzers and similar equipment has to be validated. Any change in design, composition and test procedure as well as for any use in combination with other products not approved by the manufacturer is not authorized; the user himself is responsible for such changes. The manufacturer is not liable for false results and incidents for these reasons. The manufacturer is not liable for any results by visual analysis of the patient samples.

- Only for in-vitro diagnostic use.

- All 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.

- The GenWay ELISA is only designed for qualified personnel who are familiar with good laboratory practice.

| WARNING: | Sulfuric acid irritates eyes and skin. Keep out of the reach of children. Upon contact with the eyes, rinse thoroughly with water and consult a doctor! |

12.1. Disposal Considerations

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

13. ORDERING INFORMATION

Prod. No: 40-521-475133

Toxoplasma gondii IgM µ-capture ELISA (96 Determinations)
BIBLIOGRAPHY


<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tr>
<td>CONTROL -</td>
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<td>CONTROL +</td>
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<tr>
<td>CUT OFF</td>
<td>Cut off control serum</td>
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<tr>
<td>DIL M</td>
<td>Sample diluent buffer</td>
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<tr>
<td>SOLN STOP</td>
<td>Stop solution</td>
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<tr>
<td>SUB TMB</td>
<td>TMB Substrate solution</td>
</tr>
<tr>
<td>WASH BUF 20x</td>
<td>Washing solution 20x concentrated</td>
</tr>
<tr>
<td>∑&lt;sub&gt;n&lt;/sub&gt;</td>
<td>Contains sufficient for “n” tests</td>
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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 Antigen Conjugate and TMB Substrate Solution

Assay Procedure

<table>
<thead>
<tr>
<th></th>
<th>Substrate blank (e.g. A1)</th>
<th>Negative control</th>
<th>Positive control</th>
<th>Cut-off control</th>
<th>Sample (diluted 1+100)</th>
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<td>Negative control</td>
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Cover wells with foil supplied in the kit

**Incubate for 1 h at 37°C**
Wash each well three times with 300µl of washing solution

<table>
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<tr>
<th></th>
<th>Conjugate</th>
<th>100µl</th>
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**Incubate for 1 h at 37°C**
Wash each well three times with 300µl of washing solution

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**Incubate for 30 min at room temperature in the dark**

Photometric measurement at 450 nm (reference wavelength: 620 nm)