



User's Manual

Toxoplasma gondii IgM ELISA Kit



DEIANS034



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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Toxoplasma gondii IgM Enzyme Immunoassay Kit provides materials for the quantitative and qualitative determination of IgM-class antibodies to Toxoplasma gondii in human serum and plasma (EDTA, lithium heparin or citrate plasma).

This assay is intended for research use only.

Principles of Testing

The Toxoplasma gondii IgM ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA).

This ELISA is using a RF-Sorbent.

The Rheumatoid factor (RF) is a special autoantibody form. These are autoantibodies, which are directed against the Fc fragment of human IgG.

The RF autoantibodies are mostly class IgM, but may also be class IgA, IgG or IgE.

The use of anti-human IgG antibodies in the RF-sorbent prevents false positive or false negative results.

Samples are diluted with Sample Diluent and additionally incubated with IgG-RF-Sorbent.

Microtiter wells as a solid phase are coated with inactivated Toxoplasma gondii soluble antigen (strain RH).

Diluted specimens and ready-for-use controls are pipetted into these wells. During incubation Toxoplasma gondii-specific antibodies of positive specimens and controls are bound to the immobilized antigens.

After a washing step to remove unbound sample and control material horseradish peroxidase conjugated anti-human IgM antibodies are dispensed into the wells. During a second incubation this anti-IgM conjugate binds specifically to IgM antibodies resulting in the formation of enzyme-linked immune complexes.

After a second washing step to remove unbound conjugate the immune complexes formed (in case of positive results) are detected by incubation with TMB substrate and development of a blue color. The blue color turns into yellow by stopping the enzymatic indicator reaction with sulfuric acid.

The intensity of this color is directly proportional to the amount of Toxoplasma gondii-specific IgM antibody in the specimen. The optical density at 450 nm is read using an ELISA microtiter plate reader.

Reagents And Materials Provided

- 1. Microtiterwells**, 12x8 (break apart) strips, 96 wells; Wells coated with inactivated Toxoplasma gondii soluble antigen (strain RH). (incl. 1 cover foil)
- 2. Sample Diluent ***, 1 vial, 100 mL, ready to use, colored yellow; pH 7.2 ± 0.2.
- 3. IgG-RF-Sorbent***, 1 vial, 6.5 mL, ready to use, colored yellow; Contains anti-human IgG-class antibody.
- 4. Standard (Standard 1-3)***, 3 vials, ready to use;
Concentrations: 25, 50, 100 DU/mL,
Standard 1, 2.0 mL, colored green, green cap

Standard 2, 1.0 mL, colored blue, blue cap

Standard 3, 1.0 mL, colored red, red cap

5. Neg. Control *, 1 vial, 2.0 mL, ready to use; colored yellow, yellow cap.

6. Pos. Control *, 1 vial, 1.0 mL, ready to use; colored purple, black cap.

7. Enzyme Conjugate *, 1 vial, 20 mL, ready to use, antibody to human IgM conjugated to horseradish peroxidase; colored red

8. Substrate Solution, 1 vial, 14 mL, ready to use, Tetramethylbenzidine (TMB).

9. Stop Solution, 1 vial, 14 mL, ready to use, contains 0.2 mol/L H₂SO₄, Avoid contact with the stop solution. It may cause skin irritations and burns.

10. Wash Solution *, 1 vial, 30 mL (20× concentrated for 600 mL), pH 6.5±0.1.

*** contain non-mercury preservative**

Materials Required But Not Supplied

1. A microtiter plate calibrated reader (450 nm/620 nm ± 10 nm)
2. Calibrated variable precision micropipettes
3. Incubator 37 °C
4. Manual or automatic equipment for rinsing wells
5. Vortex tube mixer
6. Freshly distilled water
7. Timer
8. Absorbent paper

Storage

When stored at 2°C to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2°C to 8°C. Microtiter wells must be stored at 2°C to 8°C. Once the foil bag has been opened, care should be taken to close it tightly again.

Opened kits retain activity for 8 weeks if stored as described above.

Specimen Collection And Preparation

Serum or plasma (EDTA, lithium heparin or citrate plasma) can be used in this assay.

In general it should be avoided to use hemolytic, icteric or lipemic specimens. For further information refer to chapter "Interferences".

1. Specimen Collection

a. Serum:

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature. Do

not centrifuge before complete clotting has occurred. Samples containing anticoagulant may require increased clotting time.

b. Plasma:

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

2. Specimen Storage and Preparation

Specimens should be capped and may be stored for up to 5 days at 2°C to 8°C prior to assaying.

Specimens held for a longer time (up to 18 months) should be frozen only once at -20°C prior to assay.

Thawed samples should be inverted several times prior to testing.

3. Specimen Dilution

Prior to assaying each specimen is first to be diluted with Sample Diluent. For the absorption of rheumatoid factor these prediluted samples then have to be incubated with IgG-RF-Sorbent.

a. Dilute each specimen 1+50 with Sample Diluent; e.g. 10 µL of specimen + 0.5 mL of Sample Diluent. Mix well.

b. Mix well the IgG-RF-Sorbent before use.

c. Dilute this prediluted sample 1+1 with IgG-RF-Sorbent; e.g. 60 µL prediluted sample + 60 µL IgG-RF-Sorbent. Mix well.

d. Let stand at room temperature for at least 15 minutes, up to a maximum of 2 hours and mix well again.

e. Take 100 µL of these pretreated samples for the ELISA.

Note: Standards and Controls are ready for use and must not be diluted!

Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

1. Wash Solution

Dilute Wash Solution 1 + 19 (e.g. 10 mL + 190 mL) with fresh and germ free redistilled water. This diluted wash solution has a pH value of 7.2 ± 0.2 .

Consumption: ~ 5 mL per determination.

Crystals in the solution disappear by warming up to 37°C in a water bath. Be sure that the crystals are completely dissolved before use.

The diluted Wash Solution is stable for 1 week at 2°C to 8°C.

2. Disposal of the Kit

The disposal of the kit must be made according to the national regulations.

3. Damaged Test Kits

In case of any severe damage to the test kit or components, CD has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

Assay Procedure

Note:

1. It is very important to bring all reagents, samples and controls to room temperature before starting the test run!
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
4. Optical density is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
5. As a general rule the enzymatic reaction is linearly proportional to time and temperature.
6. Close reagent vials tightly immediately after use to avoid evaporation and microbial contamination.
7. To avoid cross-contamination and falsely elevated results pipette samples and dispense conjugate without splashing accurately to the bottom of wells.
8. During 37°C incubation cover microtiter strips with foil to avoid evaporation.

Procedure

Prior to commencing the assay, dilute Wash Solution, prepare samples as described in point **Specimen Dilution** and establish carefully the distribution and identification plan supplied in the kit for all specimens and controls.

1. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least:
 - 1 well (e.g. A1) for the Neg. Control,
 - 4 wells (e.g. from B1 on) for the Standard 1-3
 - 1 well (e.g. F1) for the Pos. Control,

It is left to the user to determine controls and samples in duplicate.

2. Dispense
 - 100 µL of Neg. Control into well A1
 - 100 µL of Standard 1 into well B1 + C1
 - 100 µL of Standard 2 into well D1
 - 100 µL of Standard 3 into well E1
 - 100 µL of Pos. Control into well F1 and
 - 100 µL of each pretreated sample with new disposable tips into appropriate wells.
3. Cover wells with foil supplied in the kit. Incubate for **60 minutes at 37°C**.
4. Briskly shake out the contents of the wells.

Rinse the wells 5 times with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.

Important note: The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!

5. Dispense 100 µL Enzyme Conjugate into each well.
6. Incubate for 30 minutes at room temperature (20 °C to 25 °C). **Do not expose to direct sun light!**
7. Briskly shake out the contents of the wells. Rinse the wells 5 times with diluted Wash Solution (300 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
8. Add 100 µL of Substrate Solution into all wells.
9. Incubate for exactly 15 minutes at room temperature (20 °C to 25 °C) in the dark.
10. Stop the enzymatic reaction by adding 100 µL of Stop Solution to each well. Any blue color developed during the incubation turns into yellow. **Note: Highly positive samples can cause dark precipitates of the chromogen!**
11. Read the optical density at 450 nm/620 nm with a microtiter plate reader within 30 minutes after adding the Stop Solution.
12. Measure the optical density (OD) of all wells at 450 nm and record the OD values for each standard, control and sample in the distribution and identification plan. Dual wavelength reading using 620 nm as reference wavelength is recommended. Where applicable calculate the mean OD values of all duplicates.

Quality Control

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact CD.

Calculation

1. Validation of the Test Run

The test run may be considered valid provided the following criteria are met:

Neg. Control in A1: OD value lower than 0.20

Standard 1 in B1+C1 : OD value between 0.35 – 0.85

Standard 2 in D1 : OD value between 0.75 – 1.50

Standard 3 in E1 : OD value between 1.00 – 2.50

Pos. Control in F1 : OD value between 0.65 – 3.00

2. Calculation of quantitative Results

In order to obtain quantitative results in DU/mL plot the (mean) OD values of Neg. Control and Standard 1, 2, and 3 on (linear/linear) graph paper in a system of coordinates against their corresponding concentrations (0, 25, 50 and 100 DU/mL) and draw a standard calibration curve (OD values on the vertical y-axis, concentrations on the horizontal x-axis).

Read results from this standard curve employing the (mean) OD values of each specimen and control.

All validated computer programs can be used, that provide the following function: 4 PL (4 Parameter Logistics).

Note: Values of additionally (1:10, in total 1:1000) diluted samples must be multiplied by the appropriate dilution factor in order to obtain correct results! (Dilution: 1:10 = Dilution factor: 10). (See Specimen Dilution).

3. Interpretation of quantitative Results

The following values should be considered as a guideline:

NEGATIVE: < 23 DU/mL

CUT-OFF VALUE: 25 DU/mL

GREY ZONE (equivocal): 23 - 28 DU/mL

POSITIVE: > 28 DU/mL

An international Standard for Toxoplasma gondii IgM is not available, therefore the test results of the Toxoplasma gondii IgM ELISA are given in DU/mL (arbitrary units).

4. Calculation of qualitative Results

OD value of Standard 1 (Cut-off) = CO

Example: 0.47 = CO

Note: Standard 1 is used as Cut-off!

5. Interpretation of qualitative Results

NEGATIVE $\text{Mean OD}_{\text{sample}} < \text{OD}_{\text{CO}} - 10\%$

GREY ZONE $\text{OD}_{\text{CO}} - 10\% \leq \text{Mean OD}_{\text{sample}} \leq \text{OD}_{\text{CO}} + 10\%$

Repeat test 2 - 4 weeks later - with new samples

Results in the second test again in the grey zone → NEGATIVE

POSITIVE $\text{Mean OD}_{\text{sample}} > \text{OD}_{\text{CO}} + 10\%$

Detection Range

The range of the assay is between 2.182 DU/mL-100 DU/mL.

Specificity

No cross reactivity was found for A. lumbricoides, Echinococcus, E.histolytica, Fasciola, G.lamblia, Schistosoma, Strongyloides, Toxocara, and Trichinella.

Interferences

Hemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

Precautions

1. This kit is for research use only. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
4. Avoid contact with Stop Solution containing 0.2 mol/L H₂SO₄. It may cause skin irritation and burns.
5. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
6. The microplate contains snap-off strips. Unused wells must be stored at 2°C to 8°C in the sealed foil pouch and used in the frame provided.
7. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
8. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (20°C to 25°C) before starting the test. Temperature will affect the optical density readings of the assay. However, values for the samples will not be affected.
9. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
10. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
11. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
15. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
16. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.



Limitations

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the optical density values.