Toxoplasma IgM ELISA Kit

Catalog Number KA3989

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

A solid-phase enzyme immunoassay for qualitative determination of IgM antibodies to *Toxoplasma spp.* in human serum or plasma.
The kit contains reagents sufficient for 96 determinations and allows to analyze 46 unknown samples in duplicates.

Background

Toxoplasmosis is a widespread infection caused by the intracellular protozoan parasite *Toxoplasma gondii*. In most cases, toxoplasmosis is a mild or asymptomatic disease; however, in immunocompromised patients this disease may be very severe and even life-threatening. Another risk group is pregnant women in whom primary toxoplasmosis can be transfected to the fetus, causing abortion and severe malformations
The presence of IgM antibodies to *Toxoplasma gondii* indicates a primary infection, reactivation of the existing infection or reinfection with *T. gondii*. It does not differentiate active from resolving or resolved infection as *Toxoplasma gondii*-specific IgM may persist for a long time. To differentiate these stages of infection, additional testing, including IgG avidity testing, antigen detection, or PCR needs to be done.

Principle of the Assay

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated with murine monoclonal antibodies to human IgM. Antibodies from the specimen bind coated murine monoclonal antibodies to IgM on the microwell surface. Unbound material is removed by washing procedure. *Toxoplasma spp.* antigen, labelled with peroxidase enzyme, is then added into the microwells and binds to anti-*Toxoplasma* IgM if present in total IgM fixed. After subsequent washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Positivity index (PI, %) is calculated by the formula (see Calculations).Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen.

General Information

Materials Supplied

List of component

Component	Amount
Toxoplasma IgM EIA strips: polystyrene microwells coated with murine monoclonal antibodies to human IgM.	96 (8x12) wells
Control sera - (CONTROL-) dilution of preselected human serum, not containing IgM antibodies to *Toxoplasma spp.* with preservative – 0.01% Bronidox L, 0.01% 2-Methyl-4- isothiazolin-3-one-hydrochloride, colourless.	0.5 mL
Control sera + (CONTROL+) dilution of preselected human serum with high content of human IgM antibodies to *Toxoplasma spp.* with preservative – 0.01% Bronidox L, 0.01% 2-Methyl-4-isothiazolin-3-one-hydrochloride; also contains red dye.	0.2 mL
Conjugate: aqueous solution of *Toxoplasma spp.* antigen coupled with horseradish peroxidase diluted on phosphate buffered solution with casein from bovine milk and detergent (Tween-20), contains 0.1% phenol as preservative and red dye.	14 mL
EIA buffer: phosphate buffered Saline with casein from bovine milk and detergent (Tween-20), contains 0.1% phenol as preservative; contains blue dye.	14 mL
Substrate solution: ready-to-use single-component tetramethylbenzidine (TMB) solution.	14 mL
Washing solution concentrate 26x: aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative.	22 mL
Stop solution: 5.0% vol/vol solution of sulphuric acid.	14 mL
Plate sealing tape	2 slides

Storage Instruction

Store the whole kit at +2…+8°C upon receipt until the expiration date. After opening the pouch keep unused microtiter wells TIGHTLY SEALED BY ADHESIVE TAPE (INCLUDED) to minimize exposure to moisture.

Component	Stability of opened/diluted components
Toxoplasma IgM EIA strips	Until exp. date
Control sera - (CONTROL-)	2 months
Control sera + (CONTROL+)	2 months
Conjugate	Until exp. date
EIA buffer	Until exp. date
Substrate solution	Until exp. date
Washing solution concentrate 26x	Concentrate - Until exp. date Diluted washing solution – 45 days at 2-8°C or 15 days at RT

Component	Stability of opened/diluted components
Stop solution	Until exp. date
Plate sealing tape	N/A

Materials Required but Not Supplied

- ✓ Distilled or deionized water;
- ✓ Automatic or semiautomatic multichannel micropipettes, 90–250 µL, is useful but not essential;
- ✓ Calibrated micropipettes with variable volume, range volume 10–250 µL;
- ✓ Calibrated microplate photometer with 450 nm wavelength and OD measuring range 0–3.0.
- ✓ Dry thermostat or thermostat shaker for 37°C ± 2°C.

Precautions for Use

- ✓ For professional use only.
- ✓ This kit is intended for research use only.
- ✓ INFECTION HAZARD: There is no available test methods that can absolutely assure that Hepatitis B and C viruses, HIV-1/2, or other infectious agents are not present in the reagents of this kit. All human products, including human 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.
- ✓ Avoid contact with stop solution containing 5.0% H_2SO_4. It may cause skin irritation and burns.
- ✓ Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents may give false results.
- ✓ Do not use the kit beyond the expiration date.
- ✓ All indicated volumes have to be performed according to the protocol. Optimal test results are obtained only when using calibrated pipettes and microplate readers.
- ✓ Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- ✓ Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guidelines or regulations.
- ✓ Do not mix reagents from different lots.
- ✓ Replace caps on reagents immediately. Do not swap caps.
- ✓ Do not pipette reagents by mouth.
- ✓ Specimens must not contain any AZIDE compounds – they inhibit activity of peroxidase.
- ✓ Material Safety Data Sheet for this product is available upon request directly from Abnova.

Assay Protocol

Reagent Preparation

- ✓ All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18…+25°C) before use.
- ✓ All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.
- ✓ It is recommended to spin down shortly the vials with calibrators on low speed centrifuge.
- ✓ Prepare washing solution from the Washing solution concentrate 26x by 26 dilution in distilled water.

Sample Preparation

- ✓ Specimen Collection and Storage

  This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided.

  Specimens may be stored for up to 48 hours at +2…+8°C before testing. For a longer storage, the specimens should be frozen at -20°C or lower. Repeated freezing/thawing should be avoided.

Assay Procedure

- ✓ *Procedure Note*

  It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

1. Put the desired number of microstrips into the frame; allocate 4 wells for control samples: CONTROL – and CONTROL + (3 and 1 wells, resp.) and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.
2. Pipet 90 µL of EIA buffer into each well.
3. Pipet 10 µL of control samples (CONTROL – and CONTROL +) and unknown samples into the wells. Cover the wells by plate adhesive tape (included into the kit).
4. Incubate 30 minutes at 37°C and continuous shaking at 500-600 rpm.
5. Prepare washing solution by 26x dilution of washing solution concentrate 26X with distilled water. Minimal quantity of washing solution should be 250 µL per well. Wash strips 3 times
6. Dispense 100 µL of Conjugate into the wells. Cover the wells by plate adhesive tape.
7. Incubate 30 minutes at 37°C and continuous shaking at 500-600 rpm.
8. Wash the strips 5 times.
9. Dispense 100 µL of Substrate solution into the wells
10. Incubate 10–20 minutes at +18…+25°C
11. Dispense 100 µL of Stop solution into the wells.
12. Measure OD (optical density) at 450 nm.
13. Set photometer blank on air.

✓ Alternative incubation

1. Put the desired number of microstrips into the frame; allocate 4 wells for control samples: CONTROL – and CONTROL + (3 and 1 wells, resp.) and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.
2. Pipet 90 µL of EIA buffer into each well.
3. Pipet 10 µL of control samples (CONTROL – and CONTROL +) and unknown samples into the wells. Cover the wells by plate adhesive tape (included into the kit).
4. Incubate 60 minutes at 37°C.
5. Prepare washing solution by 26x dilution of washing solution concentrate 26X with distilled water. Minimal quantity of washing solution should be 250 µL per well. Wash strips 3 times
6. Dispense 100 µL of Conjugate into the wells. Cover the wells by plate adhesive tape.
7. Incubate 30 minutes at 37°C.
8. Wash the strips 5 times.
9. Dispense 100 µL of Substrate solution into the wells
10. Incubate 10–20 minutes at +18…+25°C
11. Dispense 100 µL of Stop solution into the wells.
12. Measure OD (optical density) at 450 nm.
13. Set photometer blank on air.

✓ 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.

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state, and local standards and/or laws. This is especially relevant for the use of control reagents.

For the assay to be valid, the following requirements should be met:

1. OD450 for CONTROL+ should be nlt 1 AU.
2. OD450 for CONTROL- should not be more than 0.15 AU for all replicates.
3. OD450 for any CONTROL- replicate should be within 50%-150% of the mean OD450 value for CONTROL-. If any value lies outside this range (although meets requirement #2), it should be discarded and not used for calculation of the mean OD450 value for CONTROL-.

Data Analysis

Calculation of Results

- ✓ Calculate the mean absorbance values (OD450) for CONTROL- in triplicates and each pair of samples.
- ✓ Calculate the cut-off value: (mean OD450 for CONTROL-) + 0.2
- ✓ Calculate Positivity Index (PI) for each sample: PI = mean OD450 (sample) / Cut-off

- ✓ Expected Values

  If PI value is greater than 1.1, the result is POSITIVE.

  If PI value is less than 0.9, the result is NEGATIVE.

  If PI value is between 0.9 and 1.1, the result is EQUIVOCAL.

  Such samples should be retested. If the result is equivocal again, a new sample should be obtained 2–4 weeks later and tested again. If the result remains equivocal, the sample should be considered negative.

  NOTE: the persons 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 persons should be treated with depleting adsorbents before assaying.

Performance Characteristics

- ✓ Analytical specificity

  Specificity of the test was evaluated on 93 serum specimens found negative in DiaSorin (Italy) and NovaTec (Germany). All tested specimens were found negative. Based on these data, specificity of the test is 100%

- ✓ Analytical sensitivity

  Specificity of Toxoplasma IgM ELISA Kit was evaluated using Boston Biomedica, Inc panel “Toxoplasma IgM Positive Control PTT201”. The results obtained were consistent with those obtained with Abbott EIA Toxo-IgM (lot 04667M201).

- ✓ Precision

- Intra-assay precision for two different lots (CV1, CV2) is shown below:

Serum, no	replicates	IP overage value	CV1, %	CV2, %
1	32	10.1	3.2	4.4
2	32	2.7	5.1	5.8

- Inter-assay precision is shown below:

Serum, no	duplicated	IP overage value	CV, %
1	8	9.6	4.7
2	8	2.9	6.8

Resources

References

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