Toxoplasma IgM (Toxo IgM)

ENZYME IMMUNOASSAY TEST KIT

Enzyme Linked Immunosorbent Assay (ELISA) for Qualitative Detection of Toxoplasma IgM antibody in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Toxo IgM is intended for the Qualitative detection of IgM antibodies to toxoplasma gondii infection in human serum based on capture principle. For in Vitro Diagnostic Use only.

INTRODUCTION

Toxoplasmosis is caused by the intracellular parasite Toxoplasma gondii and may be contracted by consuming contaminated meat or by contact with cat feces containing oocysts. In adolescence and adulthood, most infections are subclinical. However, if a pregnant woman contracts toxoplasmosis, it may be passed through the placenta to the fetus, resulting in congenital toxoplasmosis, which is a cause of mortality and malformation. Asymptomatic infants may develop anomalies later in life.

PRINCIPLE OF THE ASSAY

The Toxo IgM assay is based on the principle of capture of these immunoglobulins by anti-human IgM monoclonal agglutinating sera coated on the solid phase. A subsequent incubation with Toxo antigen conjugated to horseradish peroxidase binds the IgM antibodies specific for the antigen and is revealed by the addition of the TMB substrate. When the enzymatic reaction is stopped by the addition of Stop Solution, a yellow colouring forms. The colour, which is proportional to the amount of specific antibodies present in the sample, can be read in an ELISA microplate reader. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

MATERIALS AND COMPONENTS

Materials provided with the test kit:

1. Coated Microwells: Purified anti-human IgM agglutinating sera coated wells.
2. Sample Diluent: Ready to use.
3. Negative Control: Ready to use.
4. Positive Control: Ready to use.
5. Wash Buffer Concentrate (20X).
6. Enzyme Conjugate: Ready to use.
7. TMB Substrate: Ready to use.
8. Stop Solution: Ready to use.
9. Pack Insert.
10. Plate Sealer.
11. Protocol Sheet.
12. Microwell Holder.

Materials required but not provided

1) Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
2) Disposable pipette tips
3) Distilled water
4) Disposable Gloves
5) ELISA reader
6) ELISA washer

STORAGE AND STABILITY

1. **Toxo IgM** kit is stable at 2-8°C up to expiry date printed on the label.
2. Coated Microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the desiccant has changed from blue to pink at the time of opening the pouch, another coated Microwells pouch should be used.
3. Diluted Wash Buffer is stable for up to one week when stored at 2-8°C.

SPECIMEN COLLECTION & PREPARATION

1. Collect Blood specimen by venipuncture according to standard procedure.
2. Serum only should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

(1) Bring all reagents and specimen to room temperature before use.
(2) Do not pipette any material by mouth.
(3) Do not eat, drink or smoke in the area where testing is done.
(4) Use protective clothing and wear gloves when handling samples.
(5) Use absorbent sheet to cover the working area.
(6) Immediately clean up any spills with sodium hypochlorite.
(7) All specimens and controls should be considered potentially infectious and discarded appropriately.
(8) Neutralize acid containing waste before adding hypochlorite.
(9) Do not use kit after the expiry date.
(10) Do not mix components of one kit with another.
(11) Always use new tip for each specimen and reagent.
(12) Do not allow liquid from one well to mix with other wells.
(13) Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95ml of distilled or deionized water).

TEST PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding **5µl** of the test samples to **200µl** of sample diluent. **(Please do not dilute Positive Control and Negative Control, they are ready for use).** Mix well.
3. Dispense **100µl** of diluted serum samples, negative control and positive control into the appropriate wells. For the reagent blank, dispense **100µl** of sample diluent in A1 well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for **45 minutes** at **37°C**.
4. After incubation, empty the microtitre wells and wash the plate **3 times** with **350µl** of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual droplets.
5. Dispense **100µl** of enzyme conjugate to each well and incubate for **45 minutes** at **37°C**.
6. After incubation, empty the microtitre wells and wash the plate **3 times** with **350µl** of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual droplets.
7. Dispense **100µl** of TMB Substrate to each well. Incubate at room temperature (18-25°C), in the dark, for **20 minutes**.
8. Stop the reaction by adding **100 µl** of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
9. Read the optical density at 450/630 nm with a microtiter plate reader within **15 minutes.**

Add **100µl** of sample diluent in A1 Microwell as blank

⬇

Add **100µl NC & PC** and diluted samples into the respective Microwells

⬇

Apply plate sealer and incubate for **45 minutes** at **37°C**

⬇

Wash micro-wells **3 times** with **350µl** diluted wash buffer

⬇

Add **100µl** Enzyme conjugate in each well

⬇

Apply plate sealer and incubate for **45 minutes** at **37°C**

⬇

Wash micro-wells **3 times** with **350µl** diluted wash buffer

⬇

Add **100µl** substrate in each well

⬇

Apply plate sealer and incubate for **20 minutes** at **18-25°C in dark**

⬇

Add **100 µl** stop solution in each well

⬇

Read results @450nm (Ref.600-700nm) within **15 minutes**

RUN CRITERIA

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

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.2.
2. The Toxo IgM Index for Negative and Positive Control should show negative and positive results.

CALCULATION OF RESULTS

1. Calculate the average value of the absorbance of the negative control.

2. Calculate the cutoff value using the following formula:
   Cut Off OD = 2 x Mean OD of Negative Control.

3. Calculate the Toxo IgM Index using the following formula:
   Toxo IgM Index= Sample OD/ Cut Off OD.

INTERPRETATION OF THE RESULT

IgM Index Value	Result
IgM Index value <0.90	Negative
IgM Index value 0.91-1.3	Grey zone
IgM Index value >1.3	Positive

PERFORMANCE CHARACTERISTICS

The precision of the assay was evaluated by testing three different sera of eight replicates over 3 days.

The intra-assay and inter-assay C.V. are summarized below:

	Negative	Low positive	Positive
Intra-assay	4.8%	5.4%	4.2%
Inter-assay	7.9%	6.2%	5.8%

IMPORTANT NOTE

1. This assay is a temperature sensitive assay. The best temperature condition for this assay is 37°C.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication of NC, PC & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. To prevent false negative and false positive IgM test results, caused by the presence of specific IgG and rheumatoid factor (RF) in some specimens, reagents provided in this kit have been formulated to resolve these interferences. However, in specimens with extremely high RF and high autoimmune antibodies, the possibility of these interferences cannot be ruled out entirely.
2. Lipemic, hemolyzed, icteric or heat inactivated sera may cause erroneous results.
3. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

BIBLIOGRAPHY

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5. Data on file: Orchid Biomedical Systems (P) Ltd.

SYMBOL KEYS

	Temperature Limitation		Consult Instructions for use
	Manufacturer	IVD	*In vitro* Diagnostic Medical Device
	Use by	REF	Catalogue Number
	Date of Manufacture	LOT	Batch Number / Lot Number
	This side up		Contains sufficient for <n> tests
	Do not reuse		

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