

## **Toxoplasma IgM ELISA Kit**

96 Tests Kit

Enzyme Immunoassay for the  
Detection of Toxoplasma IgG Antibody  
(For In Vitro Diagnostic Use Only)  
Catalogue No. PT-Toxoplasma-IgM-96

**PISHTAZ TEB DIAGNOSTICS**

### **Introduction**

*Toxoplasma gondii* has worldwide distribution and has been identified for the first time in North African rodent named *Ctenodactylus gondii* which confer Toxoplasma species name. The parasite definitive hosts are cats and other certain felines. Toxoplasma infection in human adults proceeds to a benign self limited infection. However, their tissue cysts remain for years. The most serious form of Toxoplasma infection is Congenital Toxoplasmosis which accompanied serious fetal complication based on gestational age at the time of infection. Maternal infection during early pregnancy (first trimester) leads to abortion and still birth. If the infection occurs during second trimester, irreversible complications like intracranial calcifications, hydrocephaly, microcephaly, neurological problems, etc., will occur and affected survivors suffer from mental retardation. Toxoplasma infection during third trimester is accompanied with eye complications like chorioretinitis. Consequently, diagnosis of Toxoplasma infection during pregnancy is crucial.

Nowadays, Immunofluorescence and ELISA techniques are used for the diagnosis of anti Toxoplasma IgM and IgG antibodies.

Since anti Toxoplasma IgG antibodies are generally found in normal population, To diagnose of acute Toxoplasma infection reliably, two separate serum samples taken 10 days apart are used to demonstrate specific anti-TOXO IgG titer rising. Demonstration of specific anti-Toxoplasma IgM antibody in individual sample is the alternative serological technique for diagnosis of acute Toxoplasma infection.

The Pishtazteb (PT) Toxo- IgM- ELISA kit has high sensitivity and specificity for detection of anti-Toxoplasma IgM.

### **Test Principle**

The test principle is based on antibody capture ELISA technique in which microplate wells is coated with anti-human IgM antibody. Diluted patient serum samples are allowed to react with coated antibodies. After incubation, all serum IgM molecules will attached to the coated antibodies and unbound IgM antibodies are removed by washing. Then, to find specific anti-Toxoplasma IgM, conjugated HRP-Toxoplasma antigen added into wells which bound to their specific IgM antibody. After incubation and appropriate washing, a solution of chromogen substrate is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of Toxoplasma IgM is directly proportional to the color intensity of the test sample.

### **Materials Provided With Kit**

1. Antigen coated plate (1 plate, 96 wells): Microtiter wells coated with anti-human IgM antibodies.
2. Enzyme Conjugate (20X): 1 vial, 750  $\mu$ l volume. The solution contains HRP conjugated Toxoplasma antigen, buffer and preservative. Should be diluted 1:20 by conjugate diluent before use.
3. Conjugate diluent: 1 vial, 15 ml volume, the solution contains buffer , protein and stabilizer, just for diluting the Enzyme conjugate.
4. Sample diluent: 2 vials each contain 50 ml of diluent to dilute samples. The solution contains buffer , protein as stabilizer, ready to use.
5. Positive serum control: 1 vial, 1.5 ml solution containing protein as stabilizer, 0.05% Kathon CG as preservative and inactivated Toxoplasma IgM positive human serum.
6. Cut off serum control: 1 vial, 1.5 ml volume, containing protein as stabilizer, 0.05% Kathon CG as preservative and inactivated Toxoplasma IgM positive human serum.
7. Negative control serum: 1 vial, 2 ml volume, buffer solution containing 0.05% Kathon CG as preservative and negative for Toxoplasma IgM human serum.

8. Wash solution (20X): 1 vial, 50 ml, Phosphate Buffer Saline (PBS) containing 0.05 % Tween 20 as detergent and Kathon CG as preservative.
9. Chromogen-substrate: 1 vial, 12 ml, contains tetramethyl benzidine (TMB) and hydrogen peroxide, ready to use.
10. Stop solution: 1 vial, 12 ml, 1 molar hydrochloric acid solution.
11. Cardboard sealer.

### General Information

1. Do not mix kit reagents from different lot numbers. All kit components must be used only in original kit.
2. This kit is just for the detection of Anti-Toxoplasma IgM in human serum and plasma.
3. All reagents obtained from human sources are negative for HIV Ag, HIV and HCV antibodies. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

### Storage Conditions

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants.

### Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate the specimen (maximum 2 days at 2-8°C) and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20°C. Avoid freeze-thaw of specimen during storage. Samples suspected to microbial contamination should not be used.

### Reagents & Specimens Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: Warm the vial at 37°C to dissolve possible crystals which formed due to concentration of solution. dilute concentrated wash solution 1:20 with distilled water before use. This solution will be stable for 1 week at 2-8 °C.
3. Conjugate working preparation: Calculate the desire amount of conjugate for each test run and dilute 1:20 concentrated enzyme conjugate with conjugate diluent.

### Assay Procedure

1. Use required number of wells and keep the remaining with desiccants in tightly closed sealed bag. Consider three consecutive wells in first strip as **Blank, Positive** and **Negative control serum** respectively and the next two wells for **Cut off** control serum. Rest of wells are used for diluted samples. Test steps should be done sequentially.
2. Dilute samples 1:101 with sample diluent (dilute 10 µl of sample with 1 ml of sample diluent). Kit's control sera are ready to use and do not need any dilution.
3. Add 100 µl of each control sera (positive, negative and cut off) as well as diluted test sera into appropriate wells but nothings into blank well. Duplicate runs are suggested.
4. Seal the plate with cardboard sealer tightly. Mix gently and leave wells for 30 minutes at 37°C.
5. Remove the wells content by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times (each with 300 µl of Working wash solution).
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Add 100 µl of ready to use conjugate solution into all wells except blank.

8. Seal the plate with cardboard sealer tightly. Mix gently and incubate wells for 30 minutes at 37°C.
9. Repeat steps of 5 and 6.
10. Spike 100 µl of chromogen/ substrate solution to each well.
11. Incubate the microplate wells at room temperature and dark for 15 minutes to develop color.
12. Add 100 µl of stop solution to the wells to stop reaction.
13. Read absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available). Reference filter is highly recommended.

### Validity of the Assay

The assay is to be considered valid if:

1. The OD (450 nm) value of the **Blank** should be lower than 0.1. Higher values indicate chromogen/substrate contamination.
2. The OD value for the **Negative control serum** is lower than 0.15. Higher values indicate an inappropriate washing procedure. In such a case, check the efficiency of the washing device.
3. The **Cut off control serum** mean OD is higher than 0.15.
4. The OD value of **Positive control serum** is higher than the 0.6. Lower values indicate kit reagents decay. In such a case, check expiry date of the kit before repeating the assay.

### Result Calculation

1. Measure absorbance of controls and samples at 450 nm (Use 630 nm filter as reference filter if it's available).
2. Reduce blank OD from tests and controls OD.

To estimate Cut off value, Mean OD of Cut off control sera should be calculated:

**Cut-off value = Mean OD of Cut-off control**

To distinguish between positive and negative results the cut off index should be determined:

**Cut-off index = OD of sample/Cut-off value**

Based on above formula, Results lower than 0.9 are considered as negative and those greater than 1.1 considered as positive results. Those results between 0.9-1.1 are considered as suspected results and should be re-evaluated with fresh samples after a while.

### Results Evaluation

1. Negative results indicate absence of anti-Rubella IgM antibody.
2. Positive results should be rechecked and those which become negative should be reported as negative. Faulty washing and sampling errors may leads to first positive results.

### Performance Characteristics

#### Sensitivity

To evaluate test sensitivity, 30 positive samples confirmed by chemiluminescence and ELISA were used which all showed positive results. The PT-Toxoplasma-IgM-ELISA showed 100% sensitivity which is comparable to other diagnostic kits or methods.

#### Specificity

To evaluate test specificity, 150 negative sera are tested simultaneously with PT-Toxoplasma-IgM-ELISA and chemiluminescence method. Results displayed 148 negative and 2 positive results which in test repeat 1 became negative. According to test results the PT-Toxoplasma-IgM-ELISA showed 99% specificity.

**Test Precision**

To verify test repeatability intra- and inter assay tests were performed on positive, weakly positive and negative sera. Results are shown below:

**Intra-assay test results**

	Number of Repeats	Mean OD	SD	CV%
<b>Positive Sample</b>	10	0.06	0.004	6.6
<b>Negative Samples</b>	10	1.197	0.06	5.1
<b>Weak Positive</b>	10	0.32	0.01	3.1

**Inter-assay test results**

	Number of Repeats	Mean OD	SD	CV%
<b>Positive Sample</b>	10	0.064	0.004	6.25
<b>Negative Samples</b>	10	1.22	0.03	2.4
<b>Weak Positive</b>	10	0.34	0.011	3.2




All test were run in duplicate.

**References**

- 1-VOLK. W.A. (1982). Essential of Medical Microbiology. Second ed., pp 729, G.B. Lippincott Company, Philadelphia, New York, S. Jose, Toronto
- 2-REMINGTON. J.S. and KLEIN. J.O. (1966). Infectious diseases of the fetus and newborn infant. Sanders, Philadelphia, London, Toronto.
- 3-ENGVALL. E. and PERLMANN. P. (1971). Enzyme linked immunosorbent assay (ELISA). Quantitation of specific antibodies by enzyme-labelled anti-immunoglobulin in antigen- coated tubes. J. Immunol. 109, 129-135
- 4- ENGVALL. E. and PERLMANN. P. (1971). Enzyme linked immunosorbent assay (ELISA). Quantitative assay for immunoglobulin. J. Immunochemistry, 8, 871-874

<b>Schematic Toxoplasma-IgM ELISA Test Procedure</b>			
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**Step 1**




Reagent	Control Serum	Blank	Sample
			
Control Sera	100 µl	None	None
Diluted Sample	None	None	100 µl

Cover the microplate wells with cardboard sealer tightly, Mix gently for 15 seconds and incubate them for 30 minutes at 37°C.


**Step 2**

Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.

**Step 3**




Enzyme Conjugate Solution	100 µl 	None 	100 µl 
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Cover the microplate wells with cardboard sealer and incubate for 30 minutes at 37°C.


**Step 4**




Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.


**Step 5**

Chromogen Substrate Solution	100 µl 	100 µl 	100 µl 
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Incubate wells for 15 minutes at room temperature and dark.


**Step 6**

Stop Solution	100 µl 	100 µl 	100 µl 
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Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).