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ELISA **E**NZYME **L**INKED **I**MMUNO**S**ORBENT **A**SSAY

Microwell Method

TOXOPLASMA IgG

REF **V00064**

For in vitro Diagnostic Use

P r o d u c t I n s e r t

Enzyme Linked Immunosorbent Assay for the **quantitative** determination of IgG Antibodies to *Toxoplasma gondii* in human serum or plasma.

CE 0408 IVD

Microwell Method - 96 wells

(12 x 8-well Antigen coated Strips

Individual breakaway)

INTRODUCTION

Toxoplasma gondii is the causative agent of Toxoplasmosis. It is an obligate intracellular protozoan parasite that has been found in many species of birds, reptiles and mammals.[1] The organism can be transmitted through organ transplantation, transfusion of blood and leukocyte, contact with contaminated cat feces, and ingestion of rare or raw meats.[2,3,4,5]

In adults, infection is usually benign or asymptomatic. However, symptomatic cases including fatal cases do occur in immunosuppressed patients who has clinical or laboratory evidence of damage to the central nervous system.[6] In children, the risk of fetal infection vary according to the time of pregnancy when the mother becomes infected. Maternal infections occurring during the first trimester is less likely to pass infection to the fetus, however, if transmission occurs, severe outcomes such as spontaneous abortion and hydrocephalus are more likely. Infections acquired later in pregnancy, where most fetal transmissions occur, tends to cause less severe, but nonetheless serious congenital manifestations including cerebral calcifications and learning disabilities.[7] After infection, IgM antibodies appear as early as 5 days and decrease to low levels within a few weeks or months. IgG antibodies generally appear 1-2 weeks after infection, reaching peak levels in 6-10 weeks persisting for life.

The Toxoplasma IgG EIA Test Kit is an immunoassay for the qualitative and quantitative detection of the presence of IgG antibodies to *Toxoplasma gondii* in serum or plasma specimen. The test utilizes recombinant *Toxplasma gondii* antigens to selectively detect IgG antibodies to Toxoplasma in serum or plasma.

PRINCIPLE OF THE ASSAY

The Toxoplasma IgG EIA Test Kit is a solid phase enzyme immunoassay based on indirect principle for the qualitative and quantitative detection of IgG antibodies to Toxoplasma in human serum or plasma. The microwell plate is coated with Toxoplasma recombinant antigens. During testing, the specimen diluent and the specimens are added to the antigen coated microwell plate and then incubated. If the specimens contain IgG antibodies to Toxoplasma, it will bind to the antigens coated on the microwell plate to form immobilized antigen-Toxoplasma IgG antibody complexes. If the specimens do not contain IgG antibodies to Toxoplasma, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. The enzyme-conjugated anti-human IgG antibodies are added to the microwell plate and then incubated. The enzyme-conjugated anti-human IgG antibodies will bind to the immobilized antigen-Toxoplasma IgG antibody complexes present. After the second incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color indicating the amount of Toxoplasma IgG antibodies present in the specimens. Sulfuric acid solution is added to the microwell plate to stop the reaction producing a color change from blue to yellow. The color intensity, which corresponds to the amount of Toxoplasma IgG antibodies present in the specimens, is measured with a microplate reader at 450/630-700 nm or 450 nm.

MATERIALS PROVIDED

1. **Microwell plate**: 12x8-wells strips coated with recombinant *Toxoplasma gondii* antigens.
2. **Enzyme Conjugate**: 1 vial of 12 ml; Anti-human IgG antibody bound to peroxidase; Preservative: 0,1 % ProClin™ 300
3. **Wash Buffer conc.**: 1 vial of 50 ml; 25x conc., Tris-HCl buffer containing 0,1 % Tween 20; Preservative: 0,1 % ProClin™ 300
4. **Specimen Diluent**: 1 vial of 12 ml; Tris buffer, Preservative: 0,1 % ProClin™ 300
5. **Substrate A**: 1 vial of 8 ml; Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0,1 % ProClin™ 300
6. **Substrate B**: 1 vial of 8 mL; Buffer containing tetramethylbenzidine (TMB); Preservative: 0,1 % ProClin™ 300
7. **Stop Solution**: 1 vial of 8 ml; 0,5M Sulfuric acid
8. **Calibrators**: 4 vials of 1 ml each. Diluted human serum containing the following amounts of Toxoplasma IgG antibodies; Preservative: 0,1 % ProClin™ 300:
   1) Std. 0 IU/ml 2) Std 10 IU/ml
   3) Std. 50 IU/ml 4) Std. 200 IU/ml
9. **Plate sealer**
10. **Package Insert**

MATERIALS REQUIRED BUT NOT PROVIDED

- Freshly distilled or deionized water
- Sodium hypochlorite solution for decontamination
- Absorbent paper or paper towel
- Water bath or incubator capable of maintaining 37°C ± 2°C
- Calibrated automatic or manual microwell plate washer capable of aspirating and dispensing 350 µL/well
- Disposable gloves
- Calibrated micropipettes with disposable tips capable of dispensing 5, 50 and 100 µL
- Graduated cylinders for wash buffer dilution
- Vortex mixer for specimen mixing (optional)
- Timer
- Disposable reagent reservoirs
- Calibrated microplate reader capable of reading at 450 nm with a 630-700 nm reference filter, or reading at 450 nm without a reference filter
- Automated processor (optional)

PRECAUTIONS

- For professional in vitro diagnostic use only. Do not use after expiration date.
- Do not mix reagents from other kits with different lot numbers.
- Avoid cross contamination between reagents to ensure valid test results.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipet tip for each specimen assayed.
- Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Do not allow wells to

dry out during the assay procedure.
- Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with fingertips.
- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.

HEALTH AND SAFETY INFORMATION

- Some components of this kit contain human blood derivatives which were found to be non-reactive for the HIV-1/HIV-2/HIV-O, Syphillis and HCV antibodies as well as HBsAg. But no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.
- ProClin 300 is included as a preservative in the Conjugate, Concentrated Wash Buffer, Specimen Diluent, Substrate and Calibrators. Avoid any contact with skin or eyes.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not mouth pipette.
- Avoid any contact of the Substrate A, Substrate B, and Stop Solution with skin or mucosa. The Stop Solution contains 0,5M sulfuric acid which is a strong acid. If spills occur, wipe immediately with large amounts of water. If the acid contacts the skin or eyes, flush with large amounts of water and seek medical attention.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- Handle and dispose all specimens and materials used to perform the test as if they contained infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.
- Observe Good Laboratory Practices when handling chemicals and potentially infectious material. Discard all contaminated material, specimens and reagents of human origin after proper decontamination and by following local, state and federal regulations.
- Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

STORAGE AND STABILITY OF THE KIT

- Unopened test kits should be stored at 2-8°C upon receipt. All reagents are stable through the expiration date printed on the box. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch

and removing the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch at 2-8°C and can be used within 3 month of the opening date. Return the remaining unused strips and supplied desiccant to the original resealable pouch, firmly press the seas closure to seal the pouch completely and immediately store at 2-8°C.

- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
- Do not store Stop Solution in a shallow dish or return it the original bottle after use.

SPECIMEN COLLECTION AND PREPARATION

- The Toxoplasma IgG EIA Test Kit can be performed using only human serum or plasma collected from venipuncture whole blood.
- EDTA, sodium heparin, and ACD collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not use specimes with fibrin particles or contaminated with microbial growth.
- Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

REAGENTS PREPARATION

WASH BUFFER:

Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. Pour the contents of the bottle in a graduated cylinder and fill it with freshly distilled or deionized water to 1250 mL. It is stable for 2 weeks at 15-30°C.

Note: If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C until all crystals dissolve.

ASSAY PROCEDURE

Allow reagents and specimens to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Arrange the calibrators so that well A1 is the Blank well. From well A1, arrange the calibrators in a horizontal or vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.

1. Leave A1 as Blank well.
2. Add 100 µL of Calibrator 1 in wells B1 and C1. (Blue Reagent)
   Add 100 µL of Calibrator 2 in wells D1 and E1. (Blue Reagent)

Add 100 µL of Calibrator 3 in wells F1 and G1. (Blue Reagent)
Add 100 µL of Calibrator 4 in wells H1 and A2. (Blue Reagent)

3. Add 100 µL of Specimen Diluent to assigned wells starting at B2. The color of Specimen Diluent is green.
   Add 5 µL of specimen to assigned wells starting at B2. Then a color change from green to blue will occur to verify that the specimen has been added.
   Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C.
4. Mix gently by swirling the microwell plate on a flat bench for 30 seconds.
   Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.
5. Remove the Plate Sealer.
   Wash each well 5 times with 350 µL of Working Wash Buffer per well, then remove the liquid.
   Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried.
   Note: Improper washing may cause false positive results.
6. Add 100 µL of Conjugate to each well except for the Blank well. The color of Conjugate is red.
7. Cover the microplate plate with the Plate Sealer and incubate in a water bath or an incubator at 37°C ± 2°C for 30 minutes ± 2 minutes.
8. Repeat Step 5.
9. Add 50 µL of Substrate A to each well. (Clear Reagent)
   Add 50 µL of Substrate B to each well. (Clear Reagent)
   Then a blue color should develop in wells containing Positive specimens.
10. Mix gently then cover microwell plate with Plate Sealer and incubate in a water bath or incubator at 37°C ± 2°C for 10 minutes ± 1 minute.
11. Remove the Plate Sealer.
    Add 50 µL of Stop Solution to each well. (Clear Reagent)
    Then a yellow color should develop in wells containing Positive specimens.
12. Read at 450/630-700 nm in 30 minutes.
    Note: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450/630-700 nm for better results.

ASSAY SCHEME

1. Prepare the Working Wash Buffer by diluting the Wash Buffer concentrate 1:25.
2. Follow this scheme:

REAGENTS	A1 BLANK	CALIBRATORS	SAMPLE
Calibrators	-	100 µl	-
Sample Diluent	-	-	100 µl
Sample	-	-	5 µl
Cover strips with adhesive film.			
Incubate 30 min. at +37°C.			
Peel out the adhesive film and aspirate the reaction solution from all wells.			
Wash 5 times with 350 µl of diluted Wash Buffer, carefully aspirating off the remaining liquid.			
Enzyme Conjugate	-	100 µl	100 µl
Cover strips with adhesive film.			
Incubate 30 min. at +37°C.			
Peel out the adhesive film and aspirate the reaction solution from all wells.			
Wash 5 times with 350 µl of diluted Wash Buffer, carefully aspirating off the remaining liquid.			
Substrate A	50 µl	50 µl	50 µl
Substrate B	50 µl	50 µl	50 µl
Cover strips with a new adhesive film.			

Incubate 10 min. at +37°C., protected from light.			
Stop solution	50 µl	50 µl	50 µl
Read the absorbance of each well against A1 blanking-well at 450 nm and 630-700 nm in 30 min.			

AUTOMATED PROCESSING

Automatic EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens. Incubation times may vary depending on the processors used but do not program less incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

VALIDATION REQUIREMENTS AND QUALITY CONTROL

1. Calculate the Mean Absorbance of Calibrators 1-4 by referring to the table below.

Example of Calibrator 2 Calculation

Item	Absorbance
Calibrator 2: Well D1	0,254
Calibrator 2: Well E1	0,256
Total Absorbance of Calibrator 2	0,254 + 0,256 = 0,510
Mean Absorbance of Calibrator 2	0,510/2 = 0,255

2. Check the validation requirements below to determine if the test results are valid.

Item	Validation Requirements
Blank Well	Blank Absorbance should be < 0.050 if read at 450/630-700 nm Note: It should be < 0.100 if read at 450 nm
Calibrator 1	Mean Absorbance after subtraction of Blank Absorbance should be < 0.100
Calibrator 2	Mean Absorbance after subtraction of Blank Absorbance should be > 0.150 and < 0.450
Calibrator 3	Mean Absorbance after subtraction of Blank Absorbance should be > Calibrator 2 and < Calibrator 4
Calibrator 4	Mean Absorbance after subtraction of Blank Absorbance should be > 1.000

NOTE: The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

INTERPRETATION OF RESULTS

Qualitative

Calculate the Index Value to obtain qualitative specimen results.

1. If the test is valid, obtain Cut-Off Value by subtracting the Blank Absorbance from the Mean Absorbance of Calibrator 2. See an example of Cut-Off Value calculation below.

Item	Absorbance
Blank Absorbance: Well A1	0,001
Cut-Off Value: Mean Absorbance of Calibrator 2 – Blank Absorbance	0,255 – 0,001 = 0,254

2. Calculate the Index Value by dividing the Specimen Absorbance by the Cut-Off Value, then read the results by referring to the Interpretation of Results table below.

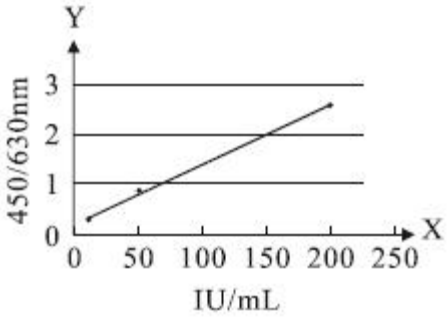
Item	Absorbance
Specimen: Well B2	0,779

Cut-Off Value	0,254
Index Value: Specimen/Cut-Off Value	0,779/0,254 = 3,067

Quantitative

Draw the calibration curve and obtain quantitative specimen results.

1. Subtract the Blank Absorbance from the Mean Absorbance of each Calibrator, then plot them on the X-axis against their concentration in IU/mL on the Y-axis on a semi-logarithmic graph paper and draw the calibration curve. Draw the best fitted line through the data points to obtain a standard curve. Refer to an example of the calibration curve at right.
   NOTE: Do not use the calibration curve at right to make any calculation. A calibration curve must be performed for each run.



2. Obtain quantitative specimen results from their absorbance by using the calibration curve.
   NOTE: Specimens that have absorbance above Calibrator 4 should be pre-diluted using Specimen Diluent and retested. The concentration must be multiplied by the dilution factor. Automated reading and calculation may be performed using linear regression function on suitable computer programs.

Interpretation of Results – Qualitative and Quantitative

Results	Qualitative	Quantitative
	Index Value	Concentration
Negative	< 0,9	< 9,0 U/mL
Positive	> 1,1	≥ 11,0 U/mL
Equivocal*	≥ 0,9 and ≤ 1,1	9,0 – 11,0 U/mL

*NOTE: For Equivocal results, the specimen should be retested. Specimens that are repeatedly Equivocal after retest should be confirmed using an alternate method. If the results remain Equivocal, collect a new specimen in two weeks. If the new specimen is Positive, the specimen is presumed to be Positive.

LIMITATIONS

1. The Toxoplasma IgG EIA Test Kit is used for the detection of IgG antibodies to Toxoplasma in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test result. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A negative test result does not exclude the possibility of exposure. Specimens Containing precipitate may give inconsistent test results.
2. As with all diagnostic tests, all results must be interpreted together with other clinical information the physician.
3. As with other sensitive immunoassays, there is the possibility that the positive result cannot be repeated due to inadequate washing from the initial test. The results may be affected due to procedural or instrument error.

PERFORMANCE CHARACTERISTICS

The calibrators are referenced to the World Health Organization International Standard Anti-Toxoplasma IgG (NIBSC code:01/600).

Sensitivity and Specificity

The Toxoplasma IgG EIA Test Kit has correctly identified specimens of a mixed titer panel when compared to a leading commercial Toxoplasma IgG EIA test. It has also been compared to a leading commercial Toxoplasma IgG EIA test using clinical specimens. The results show that the clinical sensitivity of the Toxoplasma IgG EIA Test Kit is >99.9%, and the clinical specificity is 99.0%.

Toxoplasma IgG EIA vs. Other EIA

Method		**Other EIA**		**Total Results**
Toxoplasma IgG EIA	**Results**	Positive	Negative	
	Positive	54	14	68
	Negative	0	1,314	1,314
Total Results		54	1,328	1382

Clinical Sensitivity: >99.9% (93.4-100.0%)*
Clinical Specificity: 99.0% (98.2-99.4%)*
Overall Agreement: 99.0% (98.3-99.4%)*
*95% Confidence Interval

REPRODUCIBILITY

Intra-Assay: Within-run precision has been determined by using 15 replicates of two specimens: a low positive, a medium positive and a high positive.
Inter-Assay: Between-run precision has been determined by 3 independent assays on the same three specimens: a low positive, a medium positive and a high positive. Three different lots of the Toxoplasma IgG EIA Test Kit have been tested using these specimens over a 5-day period.

Specimen	**Intra-Assay**			**Inter-Assay**		
	Mean Absorbance / Cut-Off	**Standard Deviation**	**Coefficient of Variation (%)**	**Mean Absorbance / Cut-Off**	**Standard Deviation**	**Coefficient of Variation (%)**
1	1,034	0,088	8,511	1,058	0,087	8,223
2	3,767	0,181	4,805	3,779	0,191	5,054
3	9,357	0,384	4,104	9,241	0,434	4,969

Interferences

Interferences are not observed up to concentrations of 1 mg/mL Acetaminophen, 0,2 mg/ML Gentistic Acid, 0,1 mg/mL Ancorbic Acid, 0,1% Acetosalisilyc Acid, 0,1 mg/mL Caffeine, 0,6 mg/mL Oxalic Acid, 2 mg/mL Bilirubin, 2 mg/mL Hemoglobin and 1% Ethanol. Rheumatoid Factors do not interfere with the test.
Cross-reactivity is not observed in Syphilis, HbsAg, HCV, RF and HCG positive specimens.

Dose Hook Effect
There is no dose hook effect observed with specimens up to 1000 IU.

REFERENCES

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6. Krick, JA, and Remington, JS. *Toxoplasmosis in the Adult – An Overview*. N. Engl. J. Med. (1978) 298(10):550-553.
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USED SYMBOLS

Manufacturer

Catalogue number

Use by: exp.

Temperature limitation

Batch code

Content

In vitro diagnostic medical device

Contains sufficient for <n> tests

ELISA **E**nzyme **L**inked **I**mmuno**s**orbent **A**ssay

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