



AccuDiag™ Toxoplasma IgM (Toxo IgM) ELISA Kit

REF 1102

PIC PB1102YU1

IVD See External Label 8°C 96 Tests

Toxoplasma IgM (Toxo IgM) ELISA

Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich ELISA
Detection Range	Semi-Quantitative
Sample	10 µl serum/plasma
Sensitivity	100%
Specificity	96.1%
Incubation Time	80 minutes
Shelf Life	12-14 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation Inc. (DAI) AccuDiag™ Toxoplasma IgM ELISA is intended for the semi-quantitative determination of Toxoplasma specific antibodies of the IgM type by microplate enzyme immunoassay, colorimetric.

SIGNIFICANCE AND SUMMARY

Toxoplasma gondii is an infectious parasite that infects approximately one third of the world population and is most known for causing flu-like illnesses.^{1, 2} Infection is caused by consumption of tainted meat or by contact and inadvertant ingestion of soil or food contaminated with oocytes from infected

cat feces. Most infected healthy adults are asymptomatic, or do not show or develop any symptoms since their body is able to efficiently fight the infection. However, in pregnant patients, the infection can transmit to the fetus through the placenta which may cause miscarriage, permanent neurological damage, and visual impairment.³ For this reason, it is essential to diagnose the infection early and implement treatments to protect developing fetuses.

Diagnosis of toxoplasmosis is typically by serological methods that use immunoassays to detect anti-toxoplasma immunoglobulins.⁴ Tests for immunoglobulin M (IgM) antibodies are particularly useful because IgM indicates an active, acute, or recent infection.⁵ Since Toxoplasma infection during gestation is associated with high risk to the fetus, antibodies against Toxoplasma should be screened at regular intervals throughout the course of a pregnancy to enable rapid medical intervention.

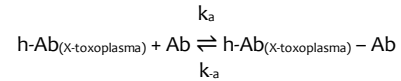
The AccuDiag™ Toxoplasma IgM ELISA Test System is a semi-quantitative test designed to produce highly sensitive and specific results with a simple and brief protocol. The test utilizes a recombinant chimeric antigen from Toxoplasma gondii conjugated to horseradish-peroxidase to detect IgM in the patient sample by a sequential sandwich type method.

ASSAY PRINCIPLE

Sequential Sandwich ELISA Method (TYPE 10):

The reagents required for the sequential ELISA assay include immobilized antibody, circulating antibody to Toxoplasma, and enzyme-linked Toxoplasma antigen.

Upon adding a sample containing the anti-toxoplasma antibody, reaction results between the anti-human IgM antibody that has been immobilized on the microwell and the antibody to form an immune-complex. The interaction is illustrated by the following equation:



Ag = Immobilized Antigen (Constant Quantity)

h-Ab_(x-toxoplasma) = Human Antibody (Variable Quantity)

h-Ab_(x-toxoplasma) - Ag = Immune Complex (Variable Quantity)

k_a = Rate Constant of Association

k_{-a} = Rate Constant of Disassociation

After the incubation time, the well is washed to separate the unbound components by aspiration and/or decantation. The enzyme linked disease-specific antigen is then added to the microwells. This conjugate binds to the immune complex that formed.

I.C. (h-IgM) + EnzAg_(Toxoplasma) ⇒ EnzAg_(Toxoplasma) - I.C. (h-IgM)

I.C. (h-IgM) = Immobilized Immune complex (Variable Quantity)

EnzAg_(Toxoplasma) = Enzyme-antigen Conjugate (Constant Quantity)

EnzAg_(Toxoplasma) - I.C. (h-IgM) = Ag-Ab Complex (Variable)

The antigen enzyme conjugate that binds to the immune complex in a second incubation is separated from unreacted material by a wash step. The enzyme activity in this fraction is directly proportional to the antibody concentration in the specimen. By utilizing a serum reference equivalent to the positive-negative cut-off value, the absorbance value can be compared to the cut-off to determine a positive or negative result.

REAGENTS

Materials provided with the test kit



A. Toxoplasma IgM Controls – 2 ml/vial

Three (3) vials of ready-to-use references for toxoplasma at positive, negative, and cut-off levels of IgM. Store at 2-8°C. A preservative has been added.

Note: The Toxoplasma IgM Cut-Off Control is traceable to the 4th International Standard for Human Antibodies to Toxoplasma gondii (NIBSC code: 13/132). The Cut-Off Value is equal to 30 IU/ml.

B. Toxoplasma IgM Enzyme Reagent – 13 ml/vial

One (1) vial of chimeric recombinant toxoplasma recombinant antigen [P22 (SAG2), P30 (SAG1), and P35 (GRA8)] horseradish peroxidases (HRP) conjugate in a buffering matrix. A preservative has been added. Store at 2-8°C.

C. IgM Antibody Coated Plate – 96 wells

One 96-well microplate coated with anti-human IgM antibody and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Serum Diluent Concentrate– 20 ml/vial

One (1) vial of concentrated serum diluent that contains buffer salts and a dye. Store at 2-8°C.

E. Wash Solution Concentrate – 20 ml/vial

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate Reagent – 12 ml/vial

One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C.

G. Stop Solution – 8 ml/vial

One (1) vial contains a strong acid (0.5 M H₂SO₄). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Avoid extended exposure to heat and light. Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on label.

Note 3: Above reagents are for a single 96-well microplate.

Materials required but not provided

1. Fixed volume or variable volume pipette capable of delivering volumes ranging from 10 to 1000 µl with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.050 ml, 0.100 ml and 0.350 ml volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

PRECAUTIONS

For In Vitro Diagnostic Use

Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication

No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

SPECIMEN COLLECTION AND PREPARATION

The specimens used should be serum or plasma from blood. The usual precautions in the collection of venipuncture samples should be observed. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin (for plasma). Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of seven (7) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.200ml of the diluted specimen is required.

QUALITY CONTROL

Each laboratory should assay controls at levels in the normal, borderline and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

REAGENT PREPARATION

1. **Serum Diluent**
Dilute contents of Serum Diluent Concentrate to 200ml (1:10 Dilution) in a suitable container with distilled or deionized water. Store at 2-8°C.
2. **Wash Buffer**
Dilute contents of wash solution concentrate to 1000 ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
3. **Patient Sample Dilution (1/100)**
For example, dispense 0.010ml (10µl) of each patient specimen into 0.990 ml (990 µl) of serum diluent or 0.0101 ml (10.1 µl) into 1 ml (1000 µl). Cover and vortex or mix thoroughly by inversion. Store at 2-8°C for up to forty-eight (48) hours.

Note: Do not use reagents that are contaminated or have bacteria growth.

ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).

****Test Procedure should be performed by a skilled individual or trained professional****

1. Format the microplates' wells for each control sample and patient specimen to be assayed in duplicate. Dilute the patient or any external control samples 1/100 (see Reagent Preparation Section). **Replace any**



unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

- Pipette 0.100 ml (100µl) of the appropriate control or diluted patient specimen into the assigned well for IgM determination.
DO NOT SHAKE THE PLATE AFTER SAMPLE ADDITION.
- Cover and incubate 30 minutes at room temperature.
- Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
- Add 350µl of wash buffer (see Reagent Preparation Section), decant (blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
- Add 0.100 ml (100µl) of the Toxoplasma IgM Enzyme Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER ENZYME ADDITION.
- Cover and incubate for thirty (30) minutes at room temperature.
- Wash the wells three (5) times with 350 µl wash buffer by repeating steps (4 & 5) as explained above.
- Add 0.100 ml (100µl) of Substrate Reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells. Do not use Substrate Reagent if it looks blue.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION.
- Incubate at room temperature for twenty (20) minutes.
- Add 0.050ml (50µl) of stop solution to each well and swirl the microplate gently for 15-20 seconds to mix. **Always add reagents in the same order to minimize reaction time differences between wells.**
- Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within fifteen (15) minutes of adding the stop solution.**

RESULTS

A Cut-Off Control (CC) and kit specific Cut-Off Factor is used to ascertain the positivity or negativity of samples. Follow the following procedure to interpret the sample results.

- Record the absorbance of all samples obtained from the printout of the microplate reader as outlined in Example 1.
- Multiply the average absorbance of the Cut-Off Control by the Cut-Off Factor to obtain the Cut-Off Value.
- Divide the average absorbance of each sample by the Cut-Off Value and multiply by 10 to obtain the relative value unit (RV).
- If RV <9, the sample is negative for Toxoplasma IgM and if RV >10, the sample is positive for Toxoplasma IgM.
- Samples with RV that fall within the range of 9-10 are considered borderline and should be retested with a new blood draw for reevaluation.
- To convert RV to IU/ml, multiply RV by 3. This calculation is accurate up to 29 RV or 87 IU/ml. Patients higher than 29 RV may not dilute linearly with respect to the cut-off value.

Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. **If such software is utilized, the validation of the software should be ascertained.**

Interpretation of Samples	
IgM < 9 RV or < 27 IU/ml	Negative

IgM 9–10 RV or 27–30 IU/ml	Borderline
IgM > 10 RV or > 30 IU/ml	Positive

EXAMPLE 1 (Cut Off Factor = 1.00)

COV = MeanCC x COF

COV = Cut-Off Value

MeanCC = Mean Absorbance of Cut-Off Control

COF = Cut-Off Factor (See Certificate of Analysis)

COV = 0.521 x 1.00 = 10.0

Sample I.D.	Abs	Mean Abs	RV	Pos/Neg
Negative	0.082	0.079	±0.521 x 10 = 1.5	Negative
	0.075			
Cut-Off	0.515	0.521	±0.521 x 10 = 10.0	Cut-Off
	0.527			
Positive	2.408	2.420	±0.521 x 10 = 46.4	Positive
	2.432			
Patient 1	0.091	0.097	±0.521 x 10 = 1.9	Negative
	0.102			
Patient 2	0.896	0.864	±0.521 x 10 = 16.6	Positive
	0.832			
Patient 3	1.841	1.855	±0.521 x 10 = 35.6	Positive
	1.869			

*The data presented in Example 1 is for illustration only and **should not** be used in lieu of a Cut-Off Control run and Cut-Off Factor with each assay. **In this example, since the Cut-Off Factor = 1.00, the average absorbance of the Cut-Off Value = 1.00 x Cut-Off Control.**

Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

- Maximum Absorbance (Positive control) > 1.5
- Positive control RV > 15
- Negative control RV < 6

RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Diagnostic Automation, Inc.

ASSAY PERFORMANCE

- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- If more than one (1) plate is used, it is recommended to repeat the Cut-Off control.
- The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious



- results.
- Use components from the same lot. No intermixing of reagents from different batches.
 - Very high concentration of anti-toxoplasma in patient specimens can contaminate samples immediately following these extreme levels. Bad duplicates are indicative of cross contamination. Repeat any sample, which follows any patient specimen with over 3.0 units of absorbance.
 - The AccuDiag™ Toxoplasma IgM ELISA Test System is a semi-quantitative assay and gives quantities of IgM only in relative units to a cut-off that is traceable to an international standard.
 - Samples, which are contaminated microbiologically, should not be used.
 - Any patient samples used in manufacturing have been heat inactivated prior to handling. However, treat all samples, including the control samples, as potentially hazardous or infectious.
 - Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed, are essential. Any deviation from Diagnostic Automation Inc's IFU may yield inaccurate results.
 - All applicable national standards, regulations, and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
 - It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
 - Risk Analysis- as required by ISO4971 - for this and other devices, made by Diagnostic Automation Inc, can be requested via email from tech1@rapidtest.com.

Presence of Toxoplasma antibodies confirmed
IgM > 30 IU/ml or 10 RV

PERFORMANCE CHARACTERISTICS

1. Precision

The precision of the AccuDiag™ Toxoplasma IgM ELISA Test System was determined on six different patient sera of varying levels. The data summary is collected in the tables below.

TABLE 1: Total Precision

Sample	Mean Value (RV)	Within-Run Precision		Total Precision (n=80)	
		SD	CV%	SD	CV%
Patient 1	30.2	1.39	4.6	2.35	7.8
Patient 2	53.7	1.38	2.6	3.22	6
Patient 3	1.8	0.09	4.9	0.24	13.1
Patient 4	16.6	0.76	4.6	2.47	14.9
Patient 5	35.7	0.88	2.5	3.66	10.3
Patient 6	7	0.4	5.8	1.03	14.7

The above data was collected using three different kits in forty assays in duplicate over twenty days.

TABLE 2: Reproducibility of Interpretation

Sample	Number Negative	Number Borderline	Number Positive
Patient 1	0/80	0/80	80/80
Patient 2	0/80	0/80	80/80
Patient 3	80/80	0/80	0/80
Patient 4	0/80	0/80	80/80
Patient 5	0/80	0/80	80/80
Patient 6	80/80	0/80	0/80

2. Sensitivity and Specificity

The sensitivity and specificity of the AccuDiag™ Toxoplasma IgM ELISA Test system was determined by measuring 135 different samples from a random population on the Diagnostic Automation Inc kit and another commercially available ELISA test. The results are tabulated below.

Diagnostic Automation Inc Interpretation

Commercial Interpretation	Positive	Negative	Total
Positive	7	0	7
Negative	5	123	128
Total	12	123	135

Diagnostic Automation Inc Interpretation	Proportion	Wilson 95% Confidence Interval
True Positives (Sensitivity)	100%	59.0 – 100.0%
True Negatives (Specificity)	96.1%	91.1 – 98.7
False Positives	3.9%	1.3 – 8.9%
False Negatives	0.0%	0.0 – 41.0%

INTERPRETATION

- Measurements and interpretation of results must be performed by a skilled individual or trained professional.**
- Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
- For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
- If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, **Diagnostic Automation Inc.** shall have no liability.
- If computer-controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
- The clinical significance of the result should be used in evaluating the possible presence of toxoplasma infection. However, **clinical inferences should not be solely based on this test** but rather as an adjunct to the clinical manifestations of the patient and other relevant tests such as Histology, nasopharyngeal swab, etc. A positive result does not indicate and does not distinguish between infection or contagiousness of toxoplasma. Similarly, a negative result does not eliminate the absence of a toxoplasma infection but rather a very low titer of antibody that may be related to the early stages of disease.

EXPECTED RANGES OF VALUES

An international standard (NIBSC Code: 13/132), from the National Institute for Biological Standards and Control (NIBSC), was used to determine expected values for the Toxoplasma IgM AccuDiag™ ELISA Test System. Based on the international standard, the following cut-off point was established.





3. Linearity

The linearity of the AccuDiag™ Toxoplasma IgM ELISA test system was tested by diluting human serum samples containing high levels of IgM against Toxoplasma (28.2 to 47.0 RV) with the serum diluent solution. The system produces excellent linearity up to 47.0 RV (141 IU/ml) and as low as 3.2 RV (9.6 IU/ml).

REFERENCES

1. Loh F., Nathan S., et al. Vaccination challenges and strategies against long-lived Toxoplasma gondii. *Vaccine* 2019. 37: 3989-4000.
2. Kochanowsky J., Koshy A. Toxoplasma gondii. *Curr Bio* 2018. 28: R761-R783.
3. Beghetto E., Spadoni A., et al. Chimeric Antigens of Toxoplasma gondii: Toward Standardization of Toxoplasmosis Serodiagnosis Using Recombinant Products. *J Clin Microbiol* 2006. 44(6): 2133-2140. doi: 10.1128/JCM.00237-06.
4. Alibakhshi A., Bandehpour M., et al. The development and evaluation of a multi-epitope antigen as a serodiagnostic marker of Toxoplasma gondii infection. *Adv Clin Exp Med* 2020. 29(6): 669-675. doi: 10.17219/acem/104554.
5. Schroeder H. Jr., Cavacini L., Structure and function of immunoglobulins. *J Allergy Clin Immunol* 2010. 125(2 Suppl 2):S41-S52. doi:10.1016/j.jaci.2009.09.046.

MANUFACTURER AND BRAND DETAILS

 <p>ISO 13485:2016 bsi ISO 13485 Quality Management for Medical Devices CERTIFIED</p>	
 <p>Diagnostic Automation/Cortez Diagnostics, Inc. 21250 Califa Street, Suite 102 and 116, Woodland Hills, California 91367 USA</p>	
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