



# **Toxoplasma gondii IgG ELISA II**

REF. 425100CE

## **INTENDED USE**

The Wampole Laboratories Toxo IgG ELISA test system is designed for the qualitative and/or quantitative determination of IgG antibodies to *Toxoplasma gondii* in human serum. The test system is intended to be used to evaluate serologic evidence of previous infection with *T. gondii*, and is for *in vitro* diagnostic use. This product is not FDA cleared (approved) for use in testing (i.e., screening) blood or plasma donors.

## **SIGNIFICANCE AND BACKGROUND**

*T. gondii* is an obligate intracellular protozoan parasite with a worldwide distribution (1,2). Although cats are the definitive host, the organism can infect almost all mammals and birds. Serological data indicates that approximately 30% of the population of most industrialized nations is chronically infected with the organism, although the prevalence varies among different populations (3).

*Toxoplasma* exists in three forms: trophozoite, cysts, and oocysts (1,2). The trophozoite is the invasive form present during the acute phase of infection. Tissue cysts are formed after multiplication of the organism within the host cell cytoplasm and may contain up to several thousand organisms. Oocysts develop in the intestinal epithelial cells of cats and are not found in other hosts. Oocysts are excreted in the feces of cats and mature after a few days.

Infection of man and other animals occurs after ingestion of either cysts in raw or undercooked meat, or mature oocysts in material contaminated with cat feces. Once ingested, the parasites are liberated from cysts or oocysts by digestive enzymes and invade the intestinal mucosa. The parasites multiply locally and are then transported to other organs forming tissue cysts which persist for the life of the host. Cysts are found predominantly in brain, heart, and skeletal muscle.

Infection with *T. gondii* is asymptomatic in the majority (80-90%) of cases (4). The most common clinical manifestation of acute toxoplasmosis in the adult is asymptomatic lymphadenopathy involving single or multiple nodes. Lymphadenopathy may be accompanied by fever, malaise, and atypical lymphocytosis symptoms which mimic infectious mononucleosis. Very rarely will more serious complications, such as encephalitis, myocarditis or pneumonitis, be seen in the normal host (1).

Although the normal host usually suffers no ill effects from infection with *T. gondii*, infection in an immunocompromised host is often fatal (5). Immunocompromised patients may develop severe disseminated toxoplasmosis or toxoplasmic encephalitis, or both. Toxoplasma is a common opportunistic infection of the central nervous system in patients with acquired immunodeficiency syndrome (AIDS) (6). Serologic evidence indicates that toxoplasmic encephalitis in AIDS patients results from reactivation of latent infections. Approximately 30% of AIDS patients who are toxoplasma antibody positive will develop toxoplasmic encephalitis (7).

When a seronegative woman becomes infected with *T. gondii* during pregnancy, the organism is often transmitted across the placenta to the fetus (1,8). The severity of infection in the fetus varies with the trimester during which the infection was acquired. Infection during the first trimester may lead to spontaneous abortion, stillbirth, or overt disease in the neonate. Infection acquired later during pregnancy is usually asymptomatic in the neonate, and may not be recognized (8).

Approximately 75% of congenitally infected newborns are symptomatic. However, nearly all children born with subclinical toxoplasmosis will develop adverse ocular or neurologic sequelae later in life. Approximately 80-85% develop chorioretinitis and some may also experience blindness or mental retardation.

A variety of serologic tests for antibodies to *T. gondii* have been used as an aid in diagnosis of acute infection and to assess previous exposure to the organism. The more widely used tests include the Sabin-Feldman dye test, direct agglutination, indirect hemagglutination, latex agglutination, indirect immunofluorescence, and ELISA (9).

## **PRINCIPLE OF THE ELISA ASSAY**

The Wampole Toxoplasma IgG ELISA test system is designed to detect IgG class antibodies to *T. gondii* in human sera. Wells of plastic microwell strips are sensitized by passive absorption with Toxoplasma antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgG ( $\gamma$  chain specific) is added to the wells and the plate is incubated. The Conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.

3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

## **MATERIALS PROVIDED**

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. Note: All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v).

PLATE	1. Plate. 96 wells configured in twelve 1x8-well strips coated with inactivated <i>T. gondii</i> antigen. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ	2. Conjugate. Conjugated (horseradish peroxidase) goat anti-human IgG ( $\gamma$ chain specific). Ready to use. One, 15 mL vial with a white cap.
CONTROL +	3. Positive Control (Human Serum). One, 0.35 mL vial with a red cap.
CAL	4. Calibrator (Human Serum). One, 0.5 mL vial with a blue cap.
CONTROL -	5. Negative Control (Human Serum). One, 0.35 mL vial with a green cap.
DILSPE	6. SAVE Diluent™ (Sample Diluent). One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate-buffered-saline, (pH 7.2 $\pm$ 0.2). Ready to use. Note: Shake Well Before Use. (Product #: 4500CC). (NOTE: This reagent may be used with any Wampole ELISA test system utilizing Product #: 4500CC). NOTE: The SAVE Diluent™ will change color in the presence of serum.
SOLN TMB	7. TMB: One 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine(TMB). Ready to use. Contains DMSO $\leq$ 15% (w).
SOLN STOP	8. Stop solution: One 15 mL bottle (red cap) containing 1M H <sub>2</sub> SO <sub>4</sub> , 0.7M HCl. Ready to use.
WASHBUF 10X	9. Wash buffer concentrate (10X): dilute 1 part concentrate + 9 parts deionized or distilled water. One 100 mL bottle (clear cap) containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). NOTE: 1X solution will have a pH of 7.2 $\pm$ 0.2.

The following components are not kit lot number dependent and may be used interchangeably with the ELISA assays: TMB, Stop Solution, and Wash Buffer.

## **Note: Kit also contains:**

1. Component list containing lot specific information is inside the kit box.
2. Package insert providing instructions for use.

## **PRECAUTIONS**

1. For *In Vitro* Diagnostic Use.
2. Normal precautions exercised in handling laboratory reagents should be followed. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, the strips should be considered **POTENTIALLY BIOHAZARDOUS MATERIALS** and handled accordingly.
4. The human serum controls are **POTENTIALLY BIOHAZARDOUS MATERIALS**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg, and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (13).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. All reagents must be allowed to reach room temperature (20-25°C) before starting the assay. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The SAVE diluent™, controls, wash buffer, and conjugate contain sodium azide at a concentration of 0.1% (w/v). Sodium azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing sodium azide.
8. The Stop Solution is TOXIC. Causes burns. Toxic by inhalation, in contact with skin and if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
9. The TMB Solution is HARMFUL. Irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. Irritating to eyes, respiratory system and skin.
11. Wipe bottom of plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Reagents from other sources or manufacturers should not be used.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with conjugate or

other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color. To help reduce the possibility of contamination, refer to Test Procedure, Substrate Incubation section to determine the amount of TMB to be used.

15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Wash solution should be collected in a disposal basin. Treat the waste solution with 10% household bleach (0.5% sodium hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Liquid waste at acid pH should be neutralized before adding to bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

#### MATERIALS REQUIRED BUT NOT PROVIDED:

- ELISA microwell reader capable of reading at a wavelength of 450nm.
- Pipettes capable of accurately delivering 10 to 200µL.
- Multichannel pipette capable of accurately delivering (50-200µL)
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or microwell washing system.
- Distilled or deionized water.
- One liter graduated cylinder.
- Serological pipettes.
- Disposable pipette tips.
- Paper towels.
- Laboratory timer to monitor incubation steps.
- Disposal basin and disinfectant. (example: 10% household bleach, 0.5% sodium hypochlorite.)

#### STORAGE CONDITIONS

1. Store the unopened kit between 2° and 8°C.
2. Coated microwell strips: Store between 2° and 8°C. Extra strips should be immediately resealed with desiccant and returned to proper storage. Strips are stable for 60 days after the envelope has been opened and properly resealed and the indicator strip on the desiccant pouch remains blue.
3. Conjugate: Store between 2° and 8°C. DO NOT FREEZE.
4. Calibrator, Positive Control and Negative Control: Store between 2° and 8°C.
5. TMB: Store between 2° and 8°C.
6. Wash Buffer concentrate (10X): Store between 2° and 25°C. Diluted wash buffer (1X) is stable at room temperature (20° to 25° C) for up to 7 days or for 30 days between 2° and 8°C.
7. SAVe Diluent™: Store between 2° and 8°C.
8. Stop Solution: Store between 2° and 25°C.

#### SPECIMEN COLLECTION

1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (10, 11). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

#### GENERAL PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20-25°C).
2. Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and returned to storage between 2° and 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Neg. Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Pos. Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVe Diluent™. NOTE: Shake Well Before Use) of the Negative Control, Calibrator, Positive Control, and each patient serum. The SAVe Diluent™ will undergo a color change confirming that the specimen has been combined with the diluent.
4. To individual wells, add 100µL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of SAVe Diluent™ to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5X.

#### A. Manual Wash Procedure:

- a. Vigorously shake out the liquid from the wells.
- b. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
- c. Repeat steps a. and b. for a total of 5 washes.
- d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (bleach) at the end of the days run.

#### B. Automated Wash Procedure:

If using an automated microwell wash system, set the dispensing volume to 300-350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

8. Add 100µL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
9. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
12. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after the addition of the Stop Solution.

#### QUALITY CONTROL

1. Each time the assay is run the Calibrator must be run in triplicate. A reagent blank, Negative Control, and Positive Control must also be included in each assay.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator and the OD values for the Positive and Negative Controls should fall within the following ranges:

	OD Range
Negative Control	≤ 0.250
Calibrator	≥ 0.300
Positive Control	≥ 0.500

- a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤ 0.9.
- b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥ 1.25.
- c. If the above conditions are not met the test should be considered invalid and should be repeated.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
5. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices.

## INTERPRETATION OF RESULTS

### A. Calculations:

#### 1. Correction Factor

A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the Calibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each lot of kit components and is printed on the Component List located in the kit box.

#### 2. Cutoff OD Value

To obtain the cutoff OD value, multiply the CF by the mean OD of the Calibrator determined above.

$$(CF \times \text{mean OD of Calibrator} = \text{cutoff OD value})$$

#### 3. Index Values or OD Ratios

Calculate the Index Value or OD Ratio for each specimen by dividing its OD value by the cutoff OD from step 2.

Example:

Mean OD of Calibrator	=	0.793
Correction Factor (CF)	=	0.25
Cut off OD	=	$0.793 \times 0.25 = 0.198$
Unknown Specimen OD	=	0.432
Specimen Index Value or OD Ratio	=	$0.432 / 0.198 = 2.18$

### B. Interpretations:

Index Values or OD ratios are interpreted as follows:

	Index Value or OD Ratio
Negative Specimens	$\leq 0.90$
Equivocal Specimens	$0.91$ to $1.09$
Positive Specimens	$\geq 1.10$

- An OD ratio  $\leq 0.90$  indicates no detectable antibody to *T. gondii*. A negative result indicates no current or previous infection with *T. gondii*. Such individuals are presumed to be susceptible to primary infection. However, specimens taken too early during a primary infection may not have detectable levels of IgG antibody. If a primary infection is suspected, another specimen should be taken in 8-14 days and tested concurrently in the same assay with the original specimen to look for seroconversion (12).
- An OD ratio  $\geq 1.10$  is positive for IgG antibody to *T. gondii*. A positive value indicates a current or previous infection with *T. gondii*.
- Specimens with OD ratio values in the equivocal range (0.91-1.09) should be retested. Specimens that remain equivocal after repeat testing should be tested by an alternate serologic procedure, such as the Wampole Laboratories indirect fluorescent antibody test system.
- To evaluate paired (acute and convalescent) sera, both samples must be tested in the same assay. If the acute specimen is negative and the convalescent specimen is positive, seroconversion has taken place and a primary infection with *T. gondii* may be indicated.

### C. Conversion of OD Ratio to IU/mL

As an option, OD ratios may be converted to IU/mL by multiplying the OD ratio by 20. IU/mL values may then be interpreted as follows:

	IU/mL
Negative Specimens	$\leq 18$
Positive Specimens	$\geq 22$
Equivocal Specimens	$18.1$ to $21.9$

Interpretation criteria for positive, negative, and equivocal specimens are as stated in section B above.

**NOTE:** The assay is linear and correlates well with the WHO Standard between 0 and 35 IU/mL. Specimens producing a result  $>35$  IU/mL should be reported as "positive", or " $>35$  IU/mL". If greater accuracy is required, the specimen must be diluted and retested. The final result may be found by multiplying the resulting IU/mL value by the dilution factor.

**Example:** Initial Result: Ratio = 2.86 = 57.3 IU/mL  
Dilute 1:4 in SAVE Diluent™; then, 1:21 as the procedure indicates  
Retest Result: Ratio = 1.46 =  $29.3 \text{ IU/mL} \times 4 = 117.3 \text{ IU/mL}$

### LIMITATION OF THE ASSAY

The antibody titer of a single serum specimen cannot be used to determine recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to demonstrate seroconversion.

- Test results should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
- Samples collected too early in the course of an infection may not have detectable levels of IgG. In such cases, a second sample may be collected after 2-7 weeks and tested concurrently with the original specimen to look for seroconversion or an IgM specific assay should be performed, such as the Wampole Laboratories Toxo IgM ELISA test system.
- A positive test for *T. gondii* IgG in neonates should be interpreted with caution since passively acquired maternal antibody can persist for up to 6 months. However, a negative test for IgG antibody in the neonate may help exclude congenital infection (12).
- The results of this test are qualitative and should be considered as either positive or negative for the presence of Toxo IgG antibodies.

## EXPECTED VALUES

Depending on age and geographic location, 20-70% of the adult population in the United States have detectable antibodies to *T. gondii* (2)

## PERFORMANCE CHARACTERISTICS

### A. Comparative Study:

The Wampole Laboratories Toxo IgG ELISA test system was compared to another ELISA procedure for detection of IgG antibodies to *T. gondii*. A total of 201 serum specimens were tested by the two procedures. These results are summarized below:

REFERENCE TOXO IgG ELISA			
		Pos.	Neg.
WAMPOLE TOXO IgG ELISA	Pos.	45	6
	Neg.	3	147
Sensitivity = 93.8% (45/48)			
Specificity = 96.1% (147/153)			

B. A study was conducted in-house to evaluate the recovery of the WHO Standard using the Wampole assay. The results of this investigation are shown below:

RESULT			
Standard	Ratio	IU/mL	INTERPRETATION
500 IU/mL	3.34	416*	Positive
250 IU/mL	2.85	208*	Positive
125 IU/mL	2.44	112*	Positive
62 IU/mL	1.78	52*	Positive
31 IU/mL	1.30	26	Positive
16 IU/mL	0.92	18.4	Equivocal
8 IU/mL	0.58	11.6	Negative
4 IU/mL	0.33	6.6	Negative
2 IU/mL	0.13	2.6	Negative

\* Specimens were initially  $>35$  IU/mL. Required additional dilutions to accurately determine the unit value.

C. Reproducibility To assess intra- and inter-assay variation of the test procedure, the Wampole Laboratories Toxo IgG ELISA was performed on four specimens with OD ratio values in the high positive, low positive, and negative ranges. Eight replicates of each sample were run on three consecutive days. The mean OD ratio and co-efficient of variation (CV) were calculated for each sample. These data are shown below:

	Inter-Assay (n=8)						Intra-Assay n=3	
	Run #1		Run #2		Run #3		Mean Ratio	CV
	Mean Ratio	CV	Mean Ratio	CV	Mean Ratio	CV		
Serum #1	3.23	4.0%	3.38	4.8%	3.32	4.1%	3.31	1.8%
Serum #2	1.90	5.7%	1.72	13.3%	1.81	7.1%	1.81	4.0%
Serum #3	1.87	6.5%	1.76	13.2%	1.53	7.5%	1.72	8.2%
Serum #4	0.46	12.0%	0.43	10.2%	0.41	8.3%	0.43	5.1%

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## ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21
2. Add diluted serum to microwell 100  $\mu$ L/well
3.  $\longrightarrow$  Incubate 20 to 30 minutes
4. Wash
5. Add Conjugate - 100  $\mu$ L/well
6.  $\longrightarrow$  Incubate 20 to 30 minutes
7. Wash
8. Add TMB 100  $\mu$ L/well
9.  $\longrightarrow$  Incubate 10 to 15 minutes
10. Add Stop Solution 50  $\mu$ L/well - Mix
11. READ



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