

TOXOPLASMA IgG ENZYME IMMUNOASSAY TEST KIT

Catalog Number: EA100998



ENZYME IMMUNOASSAY FOR THE DETECTION OF IgG ANTIBODIES TO TOXOPLASMA GONDII IN SERUM

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

PRINCIPLE OF THE TEST

Purified *Toxoplasma gondii* antigen is coated on the surface of microwells. Diluted serum sample is added to the wells, and the *Toxoplasma gondii* IgG-specific antibody, if present, binds to the antigen. All unbound materials are washed away. HRP-conjugate is added, which binds to the antibody-antigen complex. Excess HRP-conjugate is washed off and a solution of TMB Reagent is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

REAGENTS

Materials provided with the kit:

- Microtiter Wells: Toxoplasma antigen-coated wells (12x8 wells)
- Enzyme Conjugate Reagent (red color): Red cap. 1 vial (12 ml)
- Sample Diluent (green color): 1 bottle (22 ml)
- Calibrator 1: 0 IU/ml. Natural cap. (100 µL/vial)
- Calibrator 2: 32 IU/ml. Yellow cap. (100 µL/vial)
- Calibrator 3: 100 IU/ml. Red cap. (100 µL /vial)
- Calibrator 4: 300 IU/ml. Green cap. (100 µL/ vial)
- Low Control: Range stated on label. Blue cap. (100 µL/vial)
- High Control: Range stated on label. Purple cap. (100 µL/vial)
- Wash Buffer Concentrate (20x): 1 bottle (50 ml)
- TMB Reagent (One-Step): 1 vial (11 ml)
- Stop Solution: 1N HCl. Natural cap. 1 vial (11 ml)

STORAGE OF TEST KITS AND INSTRUMENTATION

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of Wash Buffer (1x). Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.

ASSAY PROCEDURE

1. Place the desired number of coated wells into the holder.
 2. Prepare 1:40 dilution of test samples, calibrators, and controls by adding 5 µl of the sample to 200 µl of Sample Diluent. Mix well.
 3. Dispense 100 µl of diluted sera, calibrators, and controls into the appropriate wells. For the reagent blank, dispense 100 µl Sample Diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well.
 4. Incubate at 37°C for 30 minutes.
 5. At the end of incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).
 6. Dispense 100 µl of Enzyme Conjugate to each well. Mix gently for 10 seconds.
 7. Incubate at 37°C for 30 minutes.
 8. Remove Enzyme Conjugate from all wells. Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).
 9. Dispense 100 µl of TMB Reagent into each well. Mix gently for 10 seconds.
 10. Incubate at 37°C for 15 minutes.
 11. Add 100 µl of Stop Solution (1N HCl) to stop reaction.
 12. **Mix gently for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.**
- Note: Make sure there are no air bubbles in each well before reading.**
13. Read O.D. at 450 nm **within 15 minutes** with a microwell reader.

CALCULATION OF RESULTS

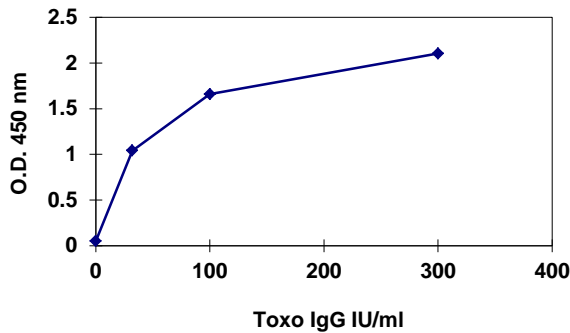
1. Calculate the mean of duplicate calibrator 2 (32 IU/ml) value x_c .
2. Calculate the mean of duplicate high control (x_h), low control (x_l) and samples (x_s).

3. Calculate the Toxoplasma IgG Index of each determination by dividing the mean values of each sample by the mean value of calibrator 2, x_c .

EXAMPLE OF TYPICAL RESULTS

This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

Toxo IgG (IU/ml)	Absorbance (450 nm)
0	0.051
32	1.040
100	1.659
300	2.103



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