

TOXOPLASMA IgM ENZYME IMMUNOASSAY TEST KIT

Catalog Number: EA100999



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.

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

FOR RESEARCH USE ONLY NOT FOR USE IN DIAGNOSTIC PROCEDURES

PRINCIPLE OF THE TEST

Purified *Toxoplasma* antigen is coated on the surface of microwells. Diluted serum sample is added to the wells, and the *Toxoplasma* IgM-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 IgM-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: purified *Toxoplasma* antigen coated wells (12 x 8 wells)
- Enzyme Conjugate Reagent (red color): Red cap. 1 vial (12 ml)
- Sample Diluent (blue color): 1 bottle (22 ml)
- Low Control: Range stated on label. Natural cap (100 µL/vial)
- Calibrator: Yellow cap. *Toxoplasma M* Index = 1 (100 µL/vial)
- High Control: Range stated on label. Red 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)

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, Low Control, High Control, and Calibrator by adding 5 µl of the sample to 200 µl of Sample Diluent. Mix well.
3. Dispense 100 µl of diluted sera, Calibrator, 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 cut-off calibrator 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 IgM Index of each determination by dividing the mean values of each sample (x) by calibrator mean value, x_c .

TECHNICAL CONSULTATION

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