



TOXO IgG EIA kit

(For in vitro diagnostic use only)

Enzyme-linked immunosorbent assay for the detection of IgG antibody to *Toxoplasma gondii*

INTENDED USE

The TOXO IgG kit is intended for use in the detection of IgG antibodies to *Toxoplasma gondii* (TOXO) infection.

SUMMARY AND PRINCIPLE OF THE TEST

Toxoplasma gondii is a coccidian parasite initially isolated in 1908 from a North African rodent - the gondii. Since then, the organism has been found in many species of birds, reptiles and mammals.

Man is infected with *Toxoplasma gondii* from various suspected sources: ingestion of infected meat, especially mutton and pork, or ingestion of soil contaminated by oocysts from domestic and feral cat. Transmission by organ transplant, transfusion or activation of quiescent infections is also documented. Congenital Toxoplasmosis is a disease with an extraordinarily wide range of manifestations; so wide in fact, that it must be considered in the differential diagnosis of nearly all types of obscure illness occurring during infancy.

Because symptoms are sometimes nonspecific (i.e., anemia, splenomegaly, jaundice, fever, hepatomegaly, adenopathy and vomiting), congenital Toxoplasmosis is easily misdiagnosed on the clinical grounds, even in sick infants who have the generalized form of the disease. Toxoplasmosis must also be considered in the differential diagnosis in any immunosuppressed patient who has clinical or laboratory evidence of damage to the central nervous system. The organism is one of the most common latent infectious agents of man through out the world .

The sensitivity, specificity, and reproducibility of enzyme-linked immunosorbent assays is comparable to other serological tests for antibody, such as immunofluorescence, complement fixation, hemagglutination and radio immunoassays .

Purified TOXO antigen is coated on the surface of microwells. Patient serum is added to wells, and the TOXO IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off and substrate and chromogen are 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 kits:

1. 8X12 well microtiter strip: 1 plate, coated with recombinant TOXO-antigen.
2. Negative Control: 1 vial

3. Positive Control: 1 vial
4. Sample Diluent: Buffer contain detergent
5. Enzyme Conjugate: HRP-conjugated-anti-IgG
6. Wash Buffer : PBS, Tween. The buffer should be diluted with distilled water 1:20 before use.
7. Substrate Solution A: urea peroxide.
8. Substrate Solution B: TMB.
9. Stop Solution: 2N Sulfuric Acid

Materials required but not provided.

1. Micropipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled or deionized water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader with 450nm wavelength
8. Timer

PRECAUTION FOR USERS

1. For in-vitro diagnostic use only.
2. Do not use kit beyond expiration date.
3. Do not mix components from kits with different lot number.
4. Avoid microbial contamination of reagents.
5. Do not pipette reagent by mouth and no smoking or eating while performing assays.
6. Wear gloves during the whole process and avoid reagents or specimen spilling-out.
7. Wipe up the spills using 5% hypochlorite solution.
8. Decontaminate all liquids or solid wastes before depositing.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum or plasma can be used in this test. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Specimen with extensive particulate should be clarified by centrifugation prior to use. Specimen frozen at -20°C or colder may be used. Avoid repeated freeze thaw.

STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C. **DO NOT FREEZE KIT COMPONENTS.** The microliter plate should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

ASSAY PROCEDURE

1. Allow all components to reach room temperature before use.
2. Dispense 100 µl of Positive Control as well as Negative Control in duplicate into respective wells. Set one blank well as background control and add 100ul Sample Diluent, and 100ul Sample Diluent and 5µl of serum or plasma samples into respective wells.
3. Place the microtiter plate into a humidified box and incubate at 37°C for 30 min.
4. Wash each well 5 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.

5. Add 100 µl of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 min. Do not add Enzyme Conjugate to the blank well.
6. Place the microtiter plate into a humidified box and incubate at 37°C for 30 min.
7. Wash each well 5 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
8. Add 50 µl of Substrate Solution A (HRP substrate) to each well, then add 50 µl of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 10 min.
9. Add one drop (50 µl) of Stop Solution to each well to stop the color reaction. Read OD values of all samples at 450 nm within 30 min.

INTERPRETATION OF RESULTS

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):

Cut Off: 0.10 + Average OD value of Negative Control

Positive: OD value is equal to or greater than the Cut Off value

Negative: OD value is less than the Cut Off value

If the OD value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual OD value measured.

LIMITATIONS OF THE ASSAY

1. Use fresh serum samples or samples frozen only once and thawed at 37° C. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield spurious results.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3. This kit is designed to measure IgG antibody in patient samples. Positive results in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the fetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
4. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended that an IgM assay be performed, or a second serum sample be obtained at a later date to be tested in parallel with the original sample to determine seroconversion .
5. The results of ELISA performed on serum from patients with immunosuppression must be interpreted with caution. The presence of IgG antibody against a particular virus or organism may not assure protection from that disease. For example, cases of reactivation of *Toxoplasma gondii* infection in immunocompromised individuals have been documented . Alternatively, certain immune individuals have been shown to have such low circulating IgG levels that they may appear negative for that antibody when tested .

RELATED READING MATERIALS

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