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User's Manual

Toxoplasma gondii IgM ELISA Kit

REF **DEIA1798-2**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

For detection of Toxoplasma gondii IgM in test samples.

General Description

Toxoplasma gondii is a species of parasitic protozoa in the genus Toxoplasma. The definitive host of T. gondii is the cat, but the parasite can be carried by many warm-blooded animals (birds or mammals, including humans). Toxoplasmosis, the disease of which T. gondii is the causative agent, is usually minor and self-limiting but can have serious or even fatal effects on a fetus whose mother first contracts the disease during pregnancy or on an immunocompromised human or cat.

Principles of Testing

This kit is designed and developed using an indirect enzyme-linked immunosorbent assay (ELISA). "Specimen, standard, (HRP-labeled) detection antibody" are added in sequence to the coated microwells pre-coated with human Toxoplasma antigen. After incubation and washing, the substrate TMB is used for color development. TMB is converted into blue under the catalysis of peroxidase and into yellow under the action of stop solution. The color is positively correlated with the human Toxoplasma IgM antibody in the sample. The absorbance (OD value) is measured at a wavelength of 450 nm using an enzyme reader to calculate the sample concentration.

Reagents And Materials Provided

1. **ELISA plate:** 96 wells 1
2. **Standard**: 300 µL/tube, 6 tubes
3. **Sample diluent**: 6 mL/bottle, 1 bottle
4. **Detection antibody (HRP)**: 10 mL/bottle, 1 bottle
5. **20× Concentrated washing solution**: 25 mL/bottle, 1 bottle
6. **Substrate A**: 6 mL/bottle, 1 bottle
7. **Substrate B**: 6 mL/bottle, 1 bottle
8. **Stop solution**: 6 mL/bottle, 1 bottle
9. Plate Sealer: 1
10. Ziplock bag: 1 piece

Notes:

1. The concentrations of the standard products are: 0.5, 1, 2, 4, 8, 16 DU/mL
2. If the normal concentration values of the samples are within the detection range provided by the kit, 50 µL of the sample can be directly loaded during the experiment. If the values of some samples exceed the maximum standard concentration, the sample can be appropriately diluted with sample diluent before the experiment.

Materials Required But Not Supplied

1. ELISA reader: 450 nm
2. Pipette and tip: 10 µL, 20 µL, 200 µL, 1000 µL
3. Thermostat: 37°C
4. Reagents: distilled water or deionized water

Storage

2-8°C (for sealed box), please do not freeze! See kit label for expiry date.

Specimen Collection And Preparation

1. **Serum**

Place whole blood sample at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20 min at 1000 ×g and collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

2. **Plasma**

EDTA-Na_2/K_2 is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000 ×g 2-8°C within 30 minutes after collection. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay. For other anticoagulant types and uses, please refer to the sample preparation guideline.

3. **Tissue homogenization**

Rinse the tissue with pre-cooled PBS (0.01M, pH=7.4) to remove residual blood (lysed red blood cells in the homogenate will affect the measurement results), weigh and mince the tissue. Add the minced tissue and the corresponding volume of PBS (generally at a weight-to-volume ratio of 1:9, for example, 1g of tissue sample corresponds to 9 mL of PBS. The specific volume can be adjusted appropriately according to experimental needs and recorded. It is recommended to add protease inhibitors to PBS) into a glass homogenizer and grind thoroughly on ice. In order to further lyse tissue cells, the homogenate can be ultrasonically disrupted or repeatedly frozen and thawed. Finally, centrifuge the homogenate at 5000 ×g for 5-10 minutes and take the supernatant for detection.

4. **Cell culture supernatant or other biological specimens**

Centrifuge at 1000 ×g for 20 minutes, take the supernatant for detection, or store the supernatant at -20°C or -80°C, but avoid repeated freezing and thawing.

Note: Hemolysis of the specimen will affect the final test results, so hemolyzed specimens are not suitable for this test.

Reagent Preparation

Allow all reagents and required number of strips to reach room temperature prior to use.

Wash Solution

Dilute Wash Solution 1 ± 19 (e.g. 10 mL ± 190 mL) with fresh and germ free redistilled water. Crystals in the

solution disappear by warming up to 37 °C in a water bath. Be sure that the crystals are completely dissolved before use. The diluted Wash Solution is stable for 4 weeks at 2 °C to 8 °C.

Assay Procedure

1. Take out the required strips from the aluminum foil bag after equilibration at room temperature (the remaining strips are sealed in a ziplock bag and returned to 4°C).
2. Set up standard wells and sample wells, and add 50 µL/well of different concentrations of standard wells.
3. Add 50 µL/well of the sample to be tested, and do not add to the blank well.
4. Add 100 µL/well of detection antibody to the standard wells and sample wells (except the blank well), seal the reaction wells with a Plate Sealer, and incubate in a 37°C incubator for 60 min.
5. Discard the liquid, pat dry, add washing solution (about 350 µL/well), let stand for 1 min, shake off the washing solution, pat dry, and repeat 5 times.
6. Add 50 µL of substrate A and B solution to each well (prepare immediately before use), and incubate at 37°C in the dark for 15 min.
7. Add 50 µL/well of stop solution and read the value within 15 min.
8. Measure the OD value of each well at a wavelength of 450 nm

Calculation

Using the OD values of the measured standard solution as the x-axis and the concentration values of the standard solution as the y-axis, plot a standard curve on graph paper or with relevant software and obtain the linear regression equation. Then, substitute the OD values of the sample into the equation to calculate the concentration of the sample.

Precision

Intra-Assay < 10%

Inter-Assay < 15%

Detection Range

0.5 DU/mL – 16 DU/mL

Sensitivity

< 0.1 DU/mL

Specificity

Specifically, no obvious cross reaction with other analogues.

Precautions

1. Adhere strictly to the specified time and temperature for incubation to ensure accurate results. Ensure all reagents reach room temperature (20-25°C) before use, and refrigerate them immediately after use.
2. Improper plate washing can lead to inaccurate results. Ensure thorough drainage of liquid from the wells before adding the substrate, and avoid letting the microwells dry out during incubation.
3. Remove any residual liquid and fingerprints from the bottom of the plate, as they can affect the OD value.
4. The substrate colorimetric solution should be colorless or very lightly colored. Do not use substrate solution that has turned blue.
5. Avoid cross-contamination of reagents and specimens to prevent erroneous results.
6. Store and incubate the materials away from direct exposure to strong light.
7. Open the sealed bag only after it has equilibrated to room temperature to prevent water droplets from condensing on the cold plate strips.
8. Ensure that no reaction reagents come into contact with bleaching solvents or the strong gases emitted by bleaching solvents, as the bleaching components can destroy the biological activity of the reaction reagents in the kit.
9. Do not use expired products.
10. Properly handle all samples and testing devices according to prescribed procedures to prevent the possibility of disease transmission.