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User's Manual

Hamster Toxoplasma (TOXO) Antibody ELISA Kit

REF **DEIA-JY2479**

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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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The Hamster Toxoplasma (TOXO) Antibody ELISA Kit is used to detect antibodies to TOXO in serum.

Principles of Testing

The ELISA kit is based on the principle of the indirect enzyme-linked immunosorbent assay (ELISA). The target antigen is pre-coated on the microplate. After adding the diluted serum to be tested, antibodies in the sample bind specifically to the pre-coated antigen. Subsequently, a secondary antibody labeled with horseradish peroxidase is added to form an antigen-antibody-enzyme complex. A colorimetric substrate for the enzyme is then added to induce color development in the reaction. Finally, the presence of corresponding antibodies in the test sample is determined by scanning with an enzyme immunoassay analyzer.

Reagents And Materials Provided

1. Pre-coated Microplate: 12 × 8. Store in a self-sealed bag with desiccant for use within 4 weeks once opened.
2. 20× Wash Buffer: 50 mL. Phosphate buffer. Dilute before use as 1× Wash buffer, store at room temperature for no more than 2 weeks before the experiment.
3. Sample Dilution Buffer: 30 mL. Ready to use.
4. Antibody-HRP Conjugate: 12 mL. Ready to use.
5. Positive Control: 1 mL. Ready to use.
6. Negative Control: 1 mL. Ready to use.
7. HRP Substrate (TMB): 12 mL. Avoid direct light.
8. Stop Solution: 5 mL. With 1.25% sodium fluoride, avoid direct contact with the eyes and skin. Ready to use.

Materials Required But Not Supplied

1. Ultra-pure deionized water or distilled water
2. V-shaped pipette trough (20-50 mL) and 96-well deep-well plate (1 mL)
3. Shaker and plate washer
4. Microplate incubator
5. Plate reader (with a 405 nm wavelength filter)
6. 10 μL and 1 mL pipettes with corresponding tips
7. 20-300 μL adjustable multichannel pipette (8 or 12 channels) with corresponding tips

Storage

The reagent basin is transported at room temperature and stored at 2-8°C. Different reagents have different

expiration dates. Microwell strips, secondary antibodies and control sera from different batches of kits should not be mixed.

Specimen Collection And Preparation

After blood collection, let it stand at room temperature for at least 1 hour. Then centrifuge at 2000g for 10 minutes. Pipette the supernatant into a sterile centrifuge tube to obtain serum or plasma samples. If the experiment will be performed within two days, the sample can be refrigerated. Otherwise, please freeze it at -20°C or below. Do not add sodium azide to the sample. Avoid repeated freezing and thawing.

Reagent Preparation

1. Before testing, remove the test kit and let it equilibrate to room temperature (approximately half an hour) before use;
2. Shake the 50 mL phosphate buffer well before pouring it into a 1000 mL reagent bottle. Add 950 mL of deionized or distilled water to prepare the washing buffer (pH 7.2-7.4). If the solution cannot be used up within two weeks, the reagent can be diluted proportionally in multiple aliquots. If crystals form in the solution, warm the reagent to 37°C to dissolve completely before preparation.

Assay Procedure

1. Sample Preparation: Arrange the serum samples in a test tube rack and use a 10 µl pipette to add 5 µl of each serum into a deep well plate. Then, add 245 µl of sample diluent into each well using a multi-channel pipette. Cover the plate and gently shake it on a shaker. Remember to change the pipette tip for each sample to avoid contamination.
2. Adding Samples: For each experiment, set up positive and negative controls in separate wells. Add 100 µl directly to the corresponding wells on the pre-coated microplate. Transfer 100 µl of the diluted samples from the deep well plate to the microplate using a multi-channel pipette. If the sample volume is low, a singlechannel pipette can be used, but keep the process brief. Cover the plate and incubate it at 37°C for 45±1 minutes.
3. Washing: After incubation, carefully remove the cover, place the microplate on a plate washer, and wash with a low flow rate washing solution 5 times, using 350 µl per well. Remove any remaining liquid from the wells.
4. Enzyme-Labeled Antibody: Pour the provided 12 ml of the working solution of the enzyme-labeled secondary antibody into a V-shaped reservoir. Add 100 µl of the working solution to each well of the microplate using a multi-channel pipette. Cover the plate and incubate it at 37°C for 45 ± 1 minutes.
5. Repeat the washing step.
6. Color Development: Place an appropriate amount of color developing reagent in a clean V-shaped reservoir and let it equilibrate to room temperature. Add 100 µl of the reagent to each well and incubate at room temperature (20-25°C) for 30 minutes.
7. Termination (Optional): If immediate reading is not possible, add 25 µl of termination reagent to each well using a multi-channel pipette to stop the color development reaction.
8. Reading: After color development, use a dual-wavelength microplate reader for immediate reading. Alternatively, add the termination reagent for 2 minutes and complete the reading within 15 minutes. (Test

wavelength 405 nm, Reference wavelength 490 nm)

Quality Control

The OD value of the negative control must be < 0.25, and the OD value of the positive control must be ≥0.60. If these standards are not met, the experiment should be repeated.

Interpretation Of Results

OD_{sample} ≥ 0.5 is considered positive;

OD_{sample} < 0.5 is considered negative.

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