Version 26-05/24

User's Manual

Chicken WNV-IgM ELISA Kit

REF **DEIA-JY2369**

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This kit allows for the determination of WNV-IgM expression in Chicken serum, and other biological fluids.

Principles of Testing

The assay kit is used to determine the WNV-IgM level in the sample. It involves coating the microtiter plate wells with purified antigen to create a solid-phase antigen. WNV-IgM is then added to the wells and combined with the solid-phase antigen. After washing and removing non-combined antibodies and other components, the combined antigen, along with HRP labeling, forms an antigen-antibody-enzyme-antigen complex. Following complete washing, a TMB substrate solution is added, resulting in the TMB substrate turning blue due to the HRP enzyme-catalyzed reaction. The reaction is terminated by the addition of a sulfuric acid solution, and the color change is measured spectrophotometrically at a wavelength of 450 nm. By comparing the results with the CUTOFF value, the presence of WNV-IgM in the sample is determined.

Reagents And Materials Provided

Materials Provided	96 determinations	Storage
User manual	1	
Closure plate membrane	2	
Sealed Bags	1	
Microelisa Stripplate	1	2-8℃
Negative Control	0.5 ml × 1 bottle	2-8℃
Positive Control	0.5 ml × 1 bottle	2-8℃
HRP-Conjugate Reagent	6 ml × 1 bottle	2-8℃
Sample Diluent	6 ml × 1 bottle	2-8℃
Chromogen Solution A	6 ml × 1 bottle	2-8℃
Chromogen Solution B	6 ml × 1 bottle	2-8℃
Stop Solution	6 ml × 1 bottle	2-8℃
Wash Solution	(20 ml × 30 fold) ×1 bottle	2-8℃

Storage

Stored at 2°C to 8°C unopened reagents will retain reacivity until expiration date.

Opened reagents must be stored at 2°C to 8°C. Microtiter wells must be stored at 2°C to 8°C. Once the foil bag has been opened, care should be taken to close it tightly again.

Opened kits retain activity for two months if stored as described above.

Specimen Collection And Preparation

1. Serum: To prepare serum, allow the blood to clot at room temperature for 10-20 minutes, then centrifuge it at 2000-3000 rpm for 20 minutes. Remove the supernatant, and if precipitation appears, centrifuge it again1..
2. Plasma: For plasma preparation, use EDTA or citrate plasma as an anticoagulant. Mix it for 10-20 minutes, then centrifuge it at 2000-3000 rpm for 20 minutes. Remove the supernatant, and if precipitation appears, centrifuge it again.
3. Urine: Collect urine using a sterile container, then centrifuge it at 2000-3000 rpm for 20 minutes. Remove the supernatant, and if precipitation appears, centrifuge it again. Similar procedures can be used for hydrothorax and cerebrospinal fluid samples.
4. Cell culture supernatant: To detect secretory components, collect the supernatant in a sterile container. Then, centrifuge it at 2000-3000 rpm for 20 minutes. Remove the supernatant and detect the composition of cells. Dilute the cell suspension with PBS (pH 7.2-7.4) to reach a concentration of 1 million cells/ml. Subject the suspension to repeated freeze-thaw cycles to release intracellular components, then centrifuge it again if precipitation appears.
5. Tissue samples: After cutting the samples, check their weight and add PBS (pH 7.2-7.4). Rapidly freeze them with liquid nitrogen, then maintain them at 2-8°C after melting. Add PBS (pH 7.4), homogenize by hand or grinders, and then centrifuge at 2000-3000 rpm for 20 minutes to remove the supernatant.
6. It's important to extract specimens as soon as possible after collection and conduct experiments promptly. If immediate experimentation isn't possible, specimens can be preserved at -20°C to avoid repeated freeze-thaw cycles.
7. Samples containing NaN3 can't be used for detection because NaN3 inhibits HRP activity.

Assay Procedure

1. Plate Setup: Each microtiter plate should be labeled with a unique number corresponding to a well and number sequence. For each plate, set two wells for the positive control, two wells for the negative control, and one well for the blank comparison (which should not have sample or HRP-Conjugate reagent added, but all other steps should be performed).
2. Adding Samples: Add 50 µl of Positive control to the positive well and 50µl of Negative control to the negative well. Add 40 µl of sample dilution to the testing sample well, followed by 10µl of the testing sample. Add the samples to the bottom of the ELISA plates' coated wells, avoiding contact with the well walls as much as possible, and gently mix.
3. Incubation: Close the plate with a closure plate membrane and incubate for 30 minutes at 37°C.
4. Prepare Wash Solution: Dilute a 30-fold (or 20-fold) wash solution with distilled water until it reaches 600 ml

and reserve it for later use.

5. Washing: Remove the closure plate membrane, discard the liquid, dry by swinging, add washing buffer to each well, let it sit for 30 seconds, then drain. Repeat this process 5 times and dry by patting.
6. Adding Enzyme: Add 50 µl of HRP-Conjugate reagent to each well, except the blank well.
7. Incubation: Repeat the incubation step (Step 3).
8. Washing: Repeat the washing process (Step 5).
9. Color: Add 50 µl of Chromogen Solution A and Chromogen Solution B to each well, avoiding light exposure, and preserve for 15 minutes at 37°C.
10. Stop the Reaction: Add 50 µl of Stop Solution to each well to halt the reaction (the blue color should change to yellow).
11. Assay: Use the blank well as the zero point and read the absorbance at 450 nm within 15 minutes after adding the Stop Solution.

Quality Control

Average of Positive control well ≥ 1.00

Average of Negative control well ≤ 0.20

Calculate Critical (CUT OFF) : Critical = the average of Negative control well + 0.15

Interpretation Of Results

WNV-IgM Negative: ODsample < Calculate Critical(CUT OFF)

WNV-IgM Positive: ODsample ≥ Calculate Critical(CUT OFF)

Precautions

1. It's crucial to strictly adhere to the usage instructions and refrain from mixing reagents from different lots.
2. Upon removal from refrigeration, the kit should be allowed to reach room temperature for 15-30 minutes before use. If there are unused ELISA plates after opening, they should be stored in a sealed bag to maintain their integrity.
3. The washing buffer may experience crystallization separation, but this can be resolved by heating the water to help dissolve it when diluting. It's important to note that washing does not affect the result of the assay.
4. The closure plate membrane is designed for single-use to prevent cross-contamination and overlapping pollution.
5. It's necessary to protect the substrate from light during storage to maintain its integrity.
6. When determining the test results, it's essential to use a microtiter plate reader as the standard. When employing dual-wavelength assays, the reference wavelength should be set at 630nm.
7. All samples, washing buffer, and any waste materials must be handled according to infectious material processing guidelines. Additionally, it's important to note that the Stop Solution is 2M sulfuric acid, requiring cautious handling to ensure safety.