Version 02-06/24

User's Manual

Mouse Anti-Hantavirus ELISA Kit

REF **DEIABL10**

Σ **48T×2**

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

The Mouse Anti-Hantavirus ELISA Kit is intended for the detection of antibodies to Hantavirus in mouse serum.

Reagents And Materials Provided

1. Microplate 2
2. Dilution Buffer 2
3. Positive Control (1×) 2
4. Negative Control (1×) 2
5. Enzyme Conjugate 2
6. Wash Buffer Concentrate (20×): 2 vials, 30 mL
7. Substrate Solution (OPD) 2
8. Substrate Dilution Buffer 2
9. Stop Solution 2
10. H_2O_2 2

Materials Required But Not Supplied

1. Pipettes and pipette tips.
2. Distilled water or deionized water.
3. Volumetric containers and pipettes for reagent preparation.
4. Paper towels or absorbent paper.
5. Multi-channel micropipettes or automated microplate washer.
6. Microplate shaker capable of 600 rpm.
7. Microplate reader capable of reading absorbance at 450 nm.

Storage

1. Dilution Buffer and Enzyme Conjugates are stored at -20°C, and other reagents are stored at 2°C to 8°C. The validity period is 12 months.
2. The foil packs containing the ELISA strips should be allowed to warm to room temperature (20 - 25°C) before opening to prevent condensation. Once opened, microtitration strips may be stored at 2-8°C until the expiration date on the label, provided that desiccated conditions are maintained.

Specimen Collection And Preparation

Obtain blood and allow clot to form. Insoluble materials should be removed by centrifugation. Remove the

serum aseptically. Serum samples should be refrigerated as soon as possible after collection. If not assayed within 48 hours, the samples should be aliquotted and frozen. Avoid repeated freezing/thawing of samples. Samples should not contain sodium azide.

Reagent Preparation

Prepare the following reagents and samples before beginning the assay procedure. All reagents and samples should be at room temperature (20 - 25°C) prior to beginning the assay and may remain at room temperature during testing. Return reagents to 2-8°C immediately after use.

Wash Buffer Concentrate (20×) - 2 vials (30 mL /vial) Contains tris buffer with surfactant. Check the Wash Concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Wash Solution is stable for 3 weeks from date of preparation if stored at 2-8°C. Therefore, dilute Wash Concentrate as needed. Dilute the Wash Concentrate 1:20 with deionized or distilled water in a clean glass or plastic screw cap container (for example: add 30 mL of wash concentrate to 570 mL of water). Mix gently by inverting several times to avoid excessive foaming. When using an automated plate washer, to ensure sufficient volume.

Assay Procedure

1. Take out the antigen-coated plate. Wells 1, 3, 5, 7, 9, and 11 in rows A-H are the antigen control wells, and wells 2, 4, 6, 8, 10, and 12 are the specific antigen wells.
2. Add negative and positive serum controls to two wells each (one antigen control well, one specific antigen well), 0.1 ml per well, add 0.1 ml diluent to each of the remaining wells, and add each serum to be tested to the adjacent well Two wells (one antigen control well, one specific antigen well), 2.5 µl each.
3. Incubation: Seal the plate with sealing film, shake to mix, and incubate at 37°C for 1 hour.
4. Washing: discard the liquid in the wells, fill each well with washing solution (200 µl), let stand for 1 minute, empty wells. Repeat 5 times and then tap microwell strips on absorbent pad or paper towel to remove excess 1× Wash Buffer.
5. Dissolve the enzyme conjugate in 12 ml of Dilution Buffer, 0.1 ml per well, seal the plate with sealing film, and incubate at 37°C for 1 hour.
6. Washing: Same as step 4.
7. Color development: Dissolve OPD in substrate buffer, add 10 µl H_2O_2 per 1 ml according to the required amount, and store the remaining solution (without adding H_2O_2) at -20°C in the dark, and dissolve it in the dark before use next time. Add 0.1 ml of diluted substrate solution (with H_2O_2 added) to each well, and place at 37°C for 10-15 minutes in the dark.
8. Terminate the reaction: add 1 drop (50 µl) of stop solution to each well
9. Use a microplate reader to read: the detection wavelength is 492 nm, the reference wavelength is 405 nm, and the ΔOD value of each well is read.

Quality Control

The positive control: ΔOD value of specific antigen reaction wells of positive control - the ΔOD value of the antigen control wells of positive control ≥ 0.2,

The negative control: ΔOD value of specific antigen reaction wells of negative control - the ΔOD value of the antigen control wells of negative control < 0.2.

If the above two conditions are met at the same time, the experiment is established; Otherwise it is not established.

Interpretation Of Results

(1) The ΔOD value of the serum to be tested in the specific antigen reaction well is ≥ 0.2

(2) The ΔOD value of the serum to be tested in the specific antigen reaction well/the ΔOD value of the serum to be tested in the antigen control reaction well ≥ 2

(3) The ΔOD value of the serum to be tested in the specific antigen reaction well/the ΔOD value of the negative control and in specific antigen reaction well ≥ 2.1

If the above three conditions are met, it will be judged as positive.

The OD value of the negative serum control in specific antigen reaction wells is calculated as 0.05 if it is lower than 0.05, and the actual OD value is calculated if it is higher than 0.05.

Precautions

1. The reagent should be shaken well before use and added vertically. Do not drop it on the well wall.
2. Result judgment must be completed within 15 minutes after the reaction is terminated.