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User's Manual

West Nile Virus Envelope Protein ELISA Kit

REF **DEIA-JY2368**

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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.

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PRODUCT INFORMATION

Intended Use

The West Nile Virus Envelope Protein ELISA Kit is an enzyme immunoassay designed to measure WNV E Protein from cell or tissue samples. It provides sufficient reagents for up to 96 tests in a 96-well plate including standard curve and unknown samples. Detection sensitivity is 31.3 ng/mL.

General Description

West Nile virus (WNV) is a single-stranded RNA virus that causes West Nile fever and is a member of the genus Flavivirus, which includes Zika virus, dengue virus, and yellow fever virus. WNV is transmitted mostly by mosquitoes, but the primary hosts of WNV are birds and the transmission cycle occurs from bird to mosquito to bird.

The WNV genome is a positive single stranded RNA encased by a nucleocapsid which is contained in a lipid bi-layered 50 nm envelope. A single polyprotein is expressed and cleaved by host and viral proteases into three structural (C, prM/M, and envelope, or E protein) and seven nonstructural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins. The WNV E protein is responsible for cellular entry of WNV. The crystal structure of the WNV E protein shows that there are three domains: a βbarrel-shaped domain I, an elongated finger-like domain II, and a C-terminal immunoglobulin-like domain III8. Domain I is glycosylated at amino acid position 154, which is critical for WNV infection. The internal fusion peptide loop in domain II allows for the trimerization of the WNV E protein as well as initiation of virus entry into cells. Domain III controls binding of WNV to host cells.

Reagents And Materials Provided

Box 1 (shipped at room temperature)

1. Anti-WNV E Protein Antibody Coated Plate: One 96-well strip plate (8 × 12).
2. Biotinylated Anti-WNV E Protein Antibody (1000×): One 10 µL vial.
3. Streptavidin-Enzyme Conjugate: One 20 µL vial.
4. Assay Diluent: One 50 mL bottle.
5. Lysis Solution: One 15 mL bottle containing 0.5% Tween 20 and 1% Triton X-100 in 10× PBS. The lysis solution was developed according to Mayo and Beckwith, 2002 and Colavita et al, 2017.
6. 10× Wash Buffer: One 100 mL bottle.
7. Substrate Solution: One 12 mL amber bottle.
8. Stop Solution: One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. WNV E Protein Standard: One 50 µL vial of 100 µg/mL WNV E Protein.

Materials Required But Not Supplied

1. Recombinant Purified WNV Samples

2. Microcentrifuge
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the WNV E Protein Standard at -80°C and the Biotinylated Anti-WNV E Protein Antibody at -20°C. Avoid multiple freeze/thaw cycles. Store all other components at 4°C.

Reagent Preparation

• 1× Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.

• Biotinylated Anti-WNV E Protein Antibody and Streptavidin Enzyme Conjugate: Immediately before use dilute the Anti-WNV E Protein Antibody and the Streptavidin Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of WNV E Protein standards in the concentration range of 0 to 2000 ng/mL into Assay Diluent (Table 1).

Standard Tubes	WNV E Protein Standard (µL)	Assay Diluent (µL)	WNV E Protein (ng/mL)
1	10	490	2000
2	250 of Tube #1	250	1000
3	250 of Tube #2	250	500
4	250 of Tube #3	250	250
5	250 of Tube #4	250	125
6	250 of Tube #5	250	62.5
7	250 of Tube #6	250	31.3
8	0	250	0

Table 1. Preparation of WNV E Protein Standards

2. Transfer 225 µL of each dilution (Standard Tubes 1-8) to a microcentrifuge tube containing 25 µL of Lysis Solution.

Preparation and Inactivation of Samples

1. (Optional) Dilute viral supernatant in culture medium as needed. For unknown samples we recommend several dilutions for each sample. Include culture medium as a negative control.
2. Transfer 225 µL of each sample to a microcentrifuge tube containing 25 µL of Lysis Solution. Vortex well.
3. Incubate 60 minutes at room temperature.

Assay Procedure

1. Add 100 µL of WNV E Protein unknown sample or standard to the Anti-E Protein Antibody Coated Plate.

Each WNV E Protein unknown sample, standard and blank should be assayed in duplicate.

2. Incubate at room temperature for 1 hour on an orbital shaker.
3. Wash microwell strips 3 times with 250 µL 1× Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1× Wash Buffer.
4. Add 100 µL of the diluted Biotinylated Anti-WNV E Protein antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above.
6. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Typical Standard Curve

The following figures demonstrate typical results with the West Nile Virus Envelope Protein ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

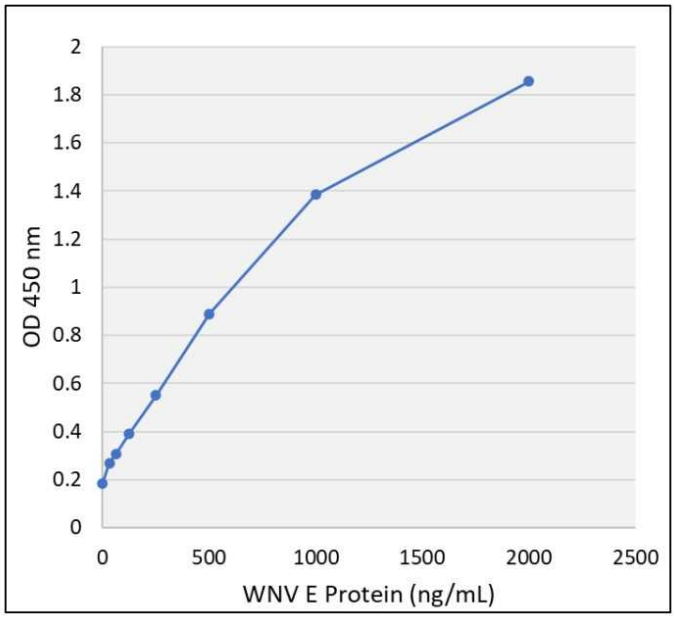

Figure 1: West Nile Virus Envelope Protein ELISA Kit Standard Curve.