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Human anti-WNV (West Nile Virus) IgM ELISA Kit

ADMEbio Catalog Number: EKFT19460HU **For Research Use Only.** Not for use in diagnostic procedures.

Product Overview

Name	Human anti-WNV (West Nile Virus) IgM ELISA Kit
Reactivity	Human
Sensitivity	Qualitative
Assay Range	Qualitative
Detection Method	Capture ELISA
Sample type(s)	Serum, Plasma, Other liquid samples

Principle of the Assay

This kit was based on Capture enzyme-linked immune-sorbent assay technology. Anti-Human-IgM□μchain□ was pre-coated onto 96-well plates. The test samples were added to the wells, unbound conjugates were washed away with wash buffer. Then added HRP- Conjugates, if there were any anti-WNV IgM in the samples, it would form a Anti-Human-IgM□μchain□- anti-WNV IgM- HRP- Conjugates complex. TMB substrates were used to visualize HRP enzymatic reaction. It was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.

Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20℃ for long term. Avoid multiple freeze-thaw cycles.

Serum: Coagulate the serum at room temperature (about 1 hour). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquot and store at -20℃.

Note: Samples to be used within 5 days may be stored at 2-8℃, otherwise samples must be stored at -20℃ (≤1 month) or -80℃(≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Material Required But Not Supplied

1. Microplate reader (wavelength: 450nm)
2.37℃ incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips

5. Clean tubes and Eppendorf tubes

6. Deionized or distilled water

Assay procedure

Remove the kit from the refrigerated environment and let it equilibrate at room temperature for 30 minutes before use

1. Label the sample wells, 3 Negative Controls, 2 Positive Controls and 1 blank well.

2. Add 50μL Negative Controls and Positive Controls to each well (except blank well).

3. Add 50μL sample dilution buffer to sample wells and then add 5μL sample serum. Gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37℃ for 30 min.

4. Remove the cover, and wash plate 3 times with Wash buffer and let the wash buffer stay in the wells for 0.5-1 minute each time. Wash the board 3 times in a row and pat dry the last time.

5. Add 50μL HRP- Conjugates to each well, except blank well.

6. Seal the plate with a cover and incubate at 37℃ for 30 min.

7. Remove the cover, and wash plate 5 times with Wash buffer and let the wash buffer stay in the wells for 0.5-1 minute each time. Wash the board 5 times in a row and pat dry the last time.

8. Add 50 μl of TMB substrate into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37℃ in dark within 15 min. And the shades of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.

9. Add 50 μl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

10. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution. (Use the blank well to set zero)(Readings are taken within 30 minutes of termination of reaction)