

CHIKV E2 (Chikungunya virus glycoprotein E2) ELISA Kit

(Do not mix reagents of different batches and different product numbers in the kit , otherwise the kit will not work properly)

Catalogue No.: EU2690

Size: 96T

Reactivity: Universal

Application: This immunoassay kit allows for the qualitative determination of CHIKV E2 in serum.

Storage: 2-8°C

Expiry Date: See kit label

Principle: Sandwich

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(96T/48T)	Storage
Micro ELISA Plate(Dismountable)	12 × 8/6× 8	2-8°C /-20°C
CHIKV E2 Positive Control	1ml×1/0.5ml×1	2-8°C
CHIKV E2 negative Control	1ml×1/0.5ml×1	2-8°C
Biotin-Anti CHIKV E2 Antibody	10ml×1/5ml×1	2-8°C(Avoid Direct Light)
HRP-Streptavidin	10ml×1/5ml×1	2-8°C(Avoid Direct Light)
Sample dilution buffer	20ml×1/10ml×1	2-8°C
TMB substrate	10ml×1/5ml×1	2-8°C(Avoid Direct Light)
Stop solution	10ml×1/5ml×1	2-8°C
Wash buffer (25X)	30ml×1/15ml×1	2-8°C
Plate Sealer	3pieces/3pieces	
Product Description	1 copy	

Principle of the Assay

This kit was based on Sandwich enzyme-linked immune-sorbent assay technology. Anti CHIKV E2 Antibody was pre-coated onto 96-well plates. The test samples were added to the wells, unbound conjugates were washed away with wash buffer. Then, biotinylated detection antibody was added to bind with CHIKV E2 conjugated on coated antibody. After washing off unbound conjugates, HRP-Streptavidin was added. After a third washing, substrate TMB was added for color development, and the presence or absence of CHIKV E2 was determined according to OD value after colorimetry on the enzyme label.

Precautions for Use

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manufacturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Material Required But Not Supplied

1. Microplate reader (wavelength: 450nm)
2. 37°C 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

Manual Washing

Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

Automated Washing

Aspirate all wells, then wash plate THREE times with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

Sample Collection and Storage

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

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

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

Wash Buffer Preparation:

Dilute 30ml concentrated wash buffer to 750ml wash buffer with deionized or distilled water and mix well. (The recommended resistivity of ultrapure water is $18\text{M}\Omega$.) Alternatively, take appropriate amount of concentrated wash buffer according to the assay requirement, then create a 20-fold dilution and mix well. Store the rest solution at $2-8^{\circ}\text{C}$.

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, 2 Negative Controls, 2 Positive Controls and 1 blank well.
2. Add $50 \mu\text{L}$ of sample diluent buffer to each well(except blank well). Then, add $50 \mu\text{L}$ of negative control, positive control or the sample to be tested into the corresponding wells respectively. Mix gently for 30 seconds.
3. Seal the plate and static incubate for 60 minutes at 37°C .
4. Remove the cover, and wash plate 3 times with Wash buffer (do not soak). Wash the plate 3 times in a row and pat dry the last time.

5. Add 100 μ L Biotin-Anti CHIKV E2 Antibody to each well, except blank well
6. Seal the plate with a cover and incubate at 37°C for 30 min.
7. 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 plate 3 times in a row and pat dry the last time.
8. Add 100ul HRP-Streptavidin into each well (except blank well), seal the plate and static incubate for 30 minutes at 37°C.
9. 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 plate 5 times in a row and pat dry the last time.
10. Add 90ul TMB substrate solution into each well, seal the plate and static incubate for 15 minutes at 37°C. And the shades of blue can be seen in the Positive Controls. Negative Controls wells show no obvious color.
11. Add 50 μ l of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.
12. 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)

Data Analysis

Calculation of the Cutoff Value

$$\text{Cutoff Value} = \text{NCx} \times 2.1$$

NCx: Mean Absorbance of Negative Control. When $\text{NCx} < 0.05$, Calculate as 0.05.

Calculation of Results

Sample with absorbance values \leq Cutoff Value is NON-REACTIVE and are considered NEGATIVE for CHIKV E2.

Sample with absorbance values $>$ Cutoff Value are considered POSITIVE for CHIKV E2.