

Canine Anti-JEV (Japanese Encephalitis Virus) IgM 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.: ECA0179

Size: 96T

Reactivity: Canine

Application: This immunoassay kit allows for the qualitative determination of anti-JEV IgM in Canine serum.

Storage: 2-8°C.

Expiry Date: see kit label

Principle: Capture

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(96T)	Storage
Micro ELISA Plate(Dismountable)	12 × 8	2-8°C/-20°C
anti-JEV IgM Positive Control (Ready-to-use)	1ml×1	2-8°C
anti-JEV IgM negative Control (Ready-to-use)	1ml×1	2-8°C
Sample dilution buffer	10ml×1	2-8°C
HRP- Conjugates (Ready-to-use)	5ml×1	2-8°C (Avoid Direct Light)
TMB substrate	10ml×1	2-8°C (Avoid Direct Light)
Stop solution	10ml×1	2-8°C
Wash buffer (25X)	30ml×1	2-8°C
Plate Sealer	3pieces	
Product Description	1 copy	

Principle of the Assay

This kit was based on capture enzyme-linked immune-sorbent assay technology for the detection of anti-JEV IgM antibodies in Canine serum samples. Anti-Canine IgM (μ chain) was pre-coated onto 96-well plates. HRP-antigen was used as the detection conjugate. Controls and test samples were added to the appropriate wells, and then HRP-antigen was added. The HRP-antigen would bind to the anti-JEV IgM that had been captured by the Anti-Canine IgM (μ chain). After washing away unbound components, TMB chromogenic substrate was added. The color development was read with a microplate reader, and the presence of anti-JEV IgM antibodies was determined according to the OD values.

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. Repeat this procedure two more times for a total of THREE washes.

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 of Concentrated Wash Buffer to 750 mL of Wash Buffer with 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 $50\mu\text{L}$ Negative Controls and Positive Controls to each well (except blank well). Add $50\mu\text{L}$ sample dilution buffer to sample wells and then add $5\mu\text{L}$ sample serum.
3. Add $50\mu\text{L}$ of HRP-Conjugat to each well except the blank well. Gently tap the plate to ensure thorough mixing. Seal the plate with a cover and incubate at 37°C for 30 min.
4. 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 $90\mu\text{L}$ of TMB substrate into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C 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 $50\mu\text{L}$ 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)

Data Analysis

- **1. Calculation of the Cutoff Value**

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

NCx: Mean Absorbance of Negative Control (When the negative mean A value is less than 0.05, it is calculated as 0.05. When the negative mean A value is greater than or equal to 0.05, it is calculated according to the actual value.)

- **2. Determination of results**

Sample with absorbance values $<$ Cutoff Value are NON-REACTIVE and are considered NEGATIVE for anti-JEV IgM.

Sample with absorbance values \geq Cutoff Value are considered POSITIVE for anti-JEV IgM.

- **3. Quality control**

The blank well (only adding TMB and Stop solution) should not be greater than 0.08.

The positive control (PC) A value was greater than 0.80.

The negative mean A value was less than 0.1.