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Rat encephalomyelitis virus (EV) ELISA Kit

96 Tests

Catalogue Number:SL1188Ra

Store all reagents at 2-8℃

Valied Perid: six months

For samples:

Serum, plasma, cell culture supernatants, body fluid and tissue homogenate.

FOR RESEARCH USE ONLY！

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS！

PLEASEREAD THROUGH ENTIRE PROCEDURE BEFORE BEGINNING

Rat encephalomyelitis virus (EV)ELISA Kit

FOR RESEARCH USE ONLY

Drug Names

Generic Name：**Rat encephalomyelitis virus (EV) ELISA Kit**

Purpose

Our Rat encephalomyelitis virus (EV) ELISA kit is to assay EV levels in Rat serum, plasma, culture media or any biological fluid.

Principle

This ELISA kit uses Sandwich-ELISA as the method. The Microelisa stripplate provided in this kit has been pre-coated with an antibody specific to EV. Standards or samples are added to the appropriate Microelisa stripplate wells and combined to the specific antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody specific for EV is added to each Microelisa stripplate well and incubated. Free components are washed away. The TMB substrate solution is added to each well. Only those wells that contain EV and HRP conjugated EV antibody will appear blue in color and then turn yellow after the addition of the stop solution. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm. The presence of EV is determined by comparing with the CUTOFF value.

Materials provided with the kit

	Materials provided with the kit	96 determinations	Storage
1	User manual	1	R.T.
2	Closure plate membrane	2	R.T.
3	Sealed bags	1	R.T.
4	Microelisa stripplate	1	2-8℃
5	Negative control	0.5ml×1 bottle	2-8℃
6	Positive control	0.5ml×1 bottle	2-8℃
7	HRP-Conjugate reagent	6ml×1 bottle	2-8℃

8	Sample diluent	6ml×1 bottle	2-8℃
9	Chromogen Solution A	6ml×1 bottle	2-8℃
10	Chromogen Solution B	6ml×1 bottle	2-8℃
11	Stop Solution	6ml×1 bottle	2-8℃
12	wash solution	20ml (30X)×1bottle	2-8℃

Sample preparation

1. **Serum preparation**

After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifugated again.

2. **Plasma preparation**

Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifugated for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifugated again.

3. **Urine samples**

Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.

4. **Cell samples**

If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X106/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifugated again.

5. **Tissue samples**

Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80℃ for future

use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4℃. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.

Notes:

1. Sample extraction and ELISA assay should be performed as soon as possible after sample collection. The samples should be extracted according to the relevant literature. If ELISA assay can not be performed immediately, samples can be stored at -20 ℃ .Repeated freeze-thaw cycles should be avoided.

2. Our kits can not be used for samples with NaN3 which can inhibit the activity of HRP.

Assay procedure

Procedure

1. In the Microelisa stripplate, leave two wells as negative control, two wells as positive control and one well empty as as blank control. Number: the sequential number, corresponding sample of the microporous hole 2 per board should set negative control and positive control 2 holes, ck 1 hole (ck hole without samples and HRP-Conjugate reagent, the rest of the same step operation)

2. Adding samples: Negative and positive control in a volume of 50μl are added to the negative and positive control wells respectively. In sample wells, 40μl Sample dilution buffer and 10μl sample are added (dilution factor is 5). Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.

3. Incubation: incubate 30 min at 37℃ after sealed with Closure plate membrane.

4. Dilution: dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T).

5. Washing: carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.

6. Add 50 μl HRP-Conjugate reagent to each well except the blank control well.

7. Incubation as described in Step 3.
8. Washing as described in Step 5.
9. Coloring: Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37℃ for 15 minutes. Please avoid light during coloring.
10. Termination: add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
11. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

Determine the result

Test effectiveness: the average value of positive control ≥1.00; the average value of negative control ≤0.10.

The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15

Negative judgement: if the OD value< CUT OFF, the sample is Rat EV negative.

Positive judgement: if the OD value ≥CUT OFF, the sample is Rat EV positive.

Notes

2. Store the kit at 4° C upon receipt.The kit should be equilibrat4ed to room temperature before the assay. Remove any unneeded strips from EV antibody -Coated plate, reseal them in zip-lock foil and keep at 4° C.
3. Precipitates may appear in concentrated washing buffer. Please heat the buffer to dissolve all the precipitates, which will not affect the results.
4. In order to avoid cross-contamination, Closure plate membranes are for one-time use only.
5. Please keep Substrate away from light.
6. All the operation should be accordance with the manufacturer's instructions strictly. The results determined by the Microelisa stripplate Reader.

7．All the samples, washing buffer and wastes should be treated as infectious agents.

8．Reagents from different lots should not be mixed.

Storage and validity

1．Storage：　2-8℃.

2．Duration: 6 months