EV71 VP1 ELISA Kit

Catalog Number KA1677

96 assays

Version: 1.5

Intended for research use only

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Introduction

Background

Enterovirus 71 (EV71) is one of the major causative agents for hand, foot and mouth disease (HFMD), is sometimes associated with severe central nervous system diseases. EV71 is notable for its etiological role in epidemics of severe neurological diseases in children. It appears to be emerging as an important virulent neurotropic enterovirus in the upcoming era of poliomyelitis eradication [1]. In 1997, in Malaysia and Japan, and in 1998 in Taiwan, there were HFMD epidemics involving sudden deaths among young children, and EV71 was isolated from the HFMD patients, including the fatal cases [2].

To date, little is known about the molecular mechanisms of host response to EV71 infection, but increases in the level of mRNAs encoding chemokines, proteins involved in protein degradation, complement proteins, and pro-apoptotic proteins have been reported [3].

Principle of the Assay

The design of this assay is based on a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific to EV71 VP1. Samples and the standard protein are pippetted into these wells. Non-specific binding and other components of the sample are removed by washing, and then HRP-conjugated monoclonal antibody specific to EV71 VP1 is added, producing an antibody- antigen-antibody “sandwich”. The final step, a TMB substrate solution is added to each well for the color development. After appropriate time of incubation, a stop solution is added and the resulting yellow colored product is measured at 450 nm with a microtiter plate reader. The increases in absorbency is directly proportional to the amount of captured EV71 VP1.

General Information

Materials Supplied

List of component

Item	Quantity	Amount
EV71 MoAb Coated Well	1 plate	8-well strips x 12
EV71 Concentrated Conjugate (100x)	1 vial	0.15 mL
EV71 Protein Standard, Lyophilized	3 vials	1.0 µg/vial
EV71 Conjugate Diluent	1 bottle	18 mL
EV71 Standard / Sample Diluent	3 bottle	12 mL/bottle
TMB Reagent	1 bottle	11 mL
Stop Solution (1N HCl)	1 bottle	11 mL
Wash Buffer (20x)	1 bottle	50 mL

1. EV71 MoAb Coated Well: one plate of 96 wells (8-well strips x 12), coated with a mouse monoclonal antibody specific to EV71 VP1.
2. EV71 Concentrated Conjugate (100x): HRP labeled mouse monoclonal anti-EV71 VP1 antibody.
3. EV71 Protein Standard, Lyophilized: Recombinant EV71 VP1 protein.

- ✓ Do not mix or interchange different reagents from various kit lots.
- ✓ All reagents should be brought to room temperature (18-25ºC) before use.

Storage Instruction

Store the kit at 2-8°C. DO NOT FREEZE.

Materials Required but Not Supplied

- ✓ Microtiter plate reader capable of measurement at or near 450nm.
- ✓ Calibrated, adjustable precision pipettes, preferably with disposable plastic tips (A manifold multi-channel pipette is desirable for large assays.)
- ✓ Distilled or deionized water
- ✓ Data analysis and graphing software
- ✓ Vortex mixer
- ✓ Polypropylene tubes for diluting and aliquoting standard
- ✓ Absorbent paper towels
- ✓ Calibrated beakers and graduated cylinders of various sizes

Precautions for Use

- ✓ This kit has been configured for research use only and is not to be used in diagnostic procedures.
- ✓ Stop solution: This reagent is an irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. Wear suitable protective clothing, gloves and eye protection. In the event of contact with eyes or skin, wash immediately with plenty of water.

Assay Protocol

Reagent Preparation

- Protein Standard

Reconstitute a vial of 1.5 µg EV71 Protein Standard, Lyophilized with 1 mL of Standard / Sample Diluent. The concentration of the reconstituted EV71 Protein Standard solution is 1500 ng/mL. Use this reconstituted standard solution to prepare a series dilution of standard as follow:

Standard point	Concentration (ng/mL)	Suggested Dilution
1	100	15x dilution from 1500 ng/mL
2	50	2 x dilution from 100 ng/mL
3	25	2 x dilution from 50 ng/mL
4	10	2.5 x dilution from 25 ng/mL
5	5.0	2 x dilution from 10 ng/mL
6	2.5	2 x dilution from 5.0 ng/mL
7	1.0	2.5 x dilution from 2.5 ng/mL
8	0	Standard / Sample Diluent only

✓ Upon reconstitution, the EV71 Protein Standard solution is very unstable, so please perform the ELISA assay immediately after reconstitution.

✓ Reconstituted standards should be discarded after use.

✓ Use a new vial of EV71 Protein Standard, Lyophilized for the next experiment.

- Wash Buffer (1x)

1. Add 50 ml of Wash Buffer (20x) to 950 ml of de-ionized water.
2. The diluted Wash Buffer(1x) is stable at 2-8ºC for 30 days. Mix well before use.

✓ Any crystals that may be present due to high salt concentration must be re-dissolved at room temperature before making the dilution.

- Working EV71 Conjugate Reagent

✓ For 3.0 ml, which is more than enough for 24 wells: Add 0.03 ml of EV71 Concentrated Conjugate (100x) to 2.97 ml of the EV71 Conjugate Diluent (1:100 dilution) and mix well.

✓ For 6.0 ml, which is more than enough for 48 wells: Add 0.06 ml of EV71 Concentrated Conjugate (100x) to 5.94 ml of the EV71 Conjugate Diluent (1:100 dilution) and mix well.

✓ For 9.0 ml, which is more than enough for 72 wells: Add 0.09 ml of EV71 Concentrated Conjugate (100x) to 8.91 ml of the EV71 Conjugate Diluent (1:100 dilution) and mix well.

✓ For 12.0 ml, which is more than enough for 96 wells: Add 0.12 ml of EV71 Conjugate Concentrate (100x) to 11.88 ml of the EV71 Conjugate Diluent (1:100 dilution) and mix well.

✓ The Working EV71 Conjugate Reagent needs to be prepared freshly every time before use.

- ✓ The amount of conjugate diluted depends on your assay size.
- ✓ DISCARD THE EXCESS AFTER USE.

Assay Procedure

1. Add 100 µL/well of EV71 Protein Standard and testing samples into appropriate wells. Mix gently for 10 seconds.
2. Incubate at 37°C for 30 minutes. No shaking.
3. Wash the plate 5 times with Wash Buffer (1x).
4. Add 100 µL of Working EV71 Conjugate Reagent to each well. Mix gently for 10 seconds.
5. Incubate at 37°C for 30 minutes. No shaking.
6. Wash the plate 5 times with Wash Buffer (1x).
7. Add 100 µL of TMB Reagent to each well. Mix gently for 10 seconds.
8. Incubate at room temperature, in the dark, for 20 minutes without shaking.
9. Add 100 µL of Stop Solution to each well. Mix gently for 30 seconds. Make sure all the blue color change to yellow color completely.
10. Read absorbance at 450 nm with a microtiter plate reader within 30 minutes.

- Directions for washing

- ✓ Fill the wells with 200 µL of Wash Buffer (1x). Let it soak for ~1 minute and then all residual wash-liquid must be drained from the wells by aspiration (taking care not to scratch the inside of the well) or decantation, followed by forceful tapping of the plate on absorbent paper. Never insert absorbent paper directly into the wells. If using an automated washer, the operating instructions for washing equipment should be carefully followed.
- ✓ Incomplete washing will adversary affects the assay and renders false results.
- ✓ It is recommended to use laboratory tape to hold plate strips to the plate frame while performing the plate washing to avoid strips coming free of the frame.

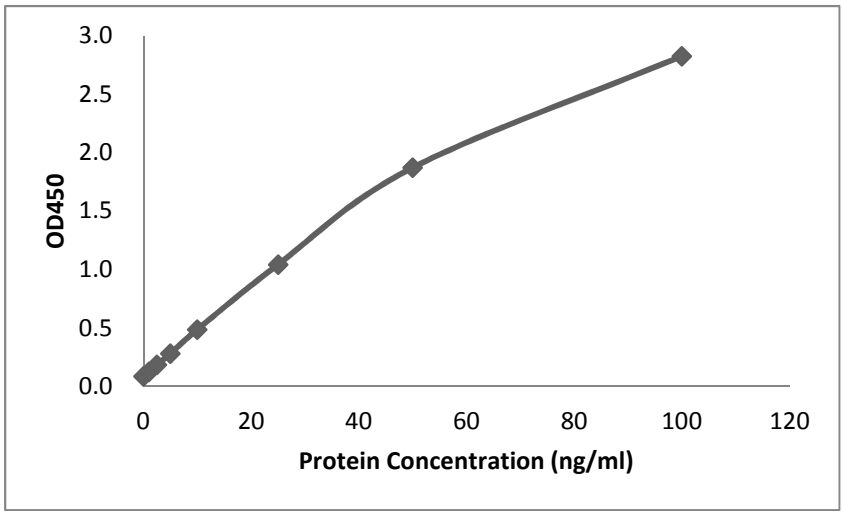
Data Analysis

Calculation of Results

The standard curve below is for illustration only and **should not be used** to calculate results in your assay.

A standard curve must be run with each assay.

EV71 VP1 Protein Standard (ng/mL)	O.D. 450 nm	CV. (%)
0	0.086	1.3
1	0.124	2.0
2.5	0.186	0.7
5	0.282	1.5
10	0.487	0.0
25	1.043	2.5
50	1.871	0.2
100	2.824	0.9



Performance Characteristics

- Sensitivity

The minimal detectable dose of EV71 VP1 was calculated to be 1 ng/mL.

- Precision

Between-Run (Inter-Assay)

EV71 VP1 Protein Standard (ng/ml)	SD	CV (%)
1	0.029	13.3
2.5	0.033	11.7
5	0.036	8.6
10	0.038	5.6
25	0.062	4.3
50	0.102	4.2
100	0.035	1.1

(N=4)

Resources

References

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3. Identification of genes involved in the host response to enterovirus 71 infection. Shin-Ru Shih, Victor Stollar, Jing-Yi Lin, Shih-Cheng Chang, Guang-Wu Chen, and Mei-Ling Li. J Neurovirol. 2004 October; 10(5): 293–304. doi: 10.1080/13550280490499551.

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