EV71 VP1 ELISA Kit

Catalog Number KA1677

96 assays

Version: 3.8

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

Component	Amount
EV71 Ab Coated Well	96 well plate (12 x 8 strips)
EV71-VPI Standard, Lyophilized	3 vials
Conjugate Concentrate (100x)	0.15 mL
Conjugate Diluent	18 mL
Standard / Sample Diluent	12 mL x 3
TMB Reagent	11 mL
Stop Solution (1 N HCl)	11 mL
Wash Buffer (20x)	50 mL

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 450 nm.
- ✓ 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

1. All reagents should be brought to room temperature (18-25°C) before use.
2. To prepare Wash Buffer (1X)
   Add 50 mL of Wash Buffer (20X) to 950 mL of DI water. The diluted Wash Buffer is stable at 2-8°C for 30 days. Mix well before use.
   Note: Any crystals that may be present due to high salt concentration must be redissolved at room temperature before making the dilution.
3. To prepare EV71 Standards
   Reconstitute the Lyophilized EV71 Standard with 1.0 mL of EV71 Standard / Sample Diluent. After reconstitution, the concentration of EV71 Standard is 1,500 ng/mL.
4. Further dilution shall be prepared according to the following table.

Standard	EV71 Conc. (ng/mL)	Dilution with Standard/Sample Diluent
1	0	Diluent only
2	1.0	1:1,500
3	2.5	1:600
4	5.0	1:300
5	10	1:150
6	25	1:60
7	50	1:30
8	100	1:15

Note:

i. The reconstituted EV71 Standard is stable at room temperature (18-25°C) for 4 hours. Discard the excess after use.

ii. Reconstituted a new vial of Lyophilized EV71 Standard for the next experiment.

5. To prepare Working Conjugate Reagent
   Dilute Conjugate Concentrate (100X) to Working Conjugate Reagent (1X) with Conjugate Diluent. The amount of conjugate diluted depends on your assay size.

Assay Procedure

(Incubation: 37°C 30' + 37°C 30' + R.T. 20')

1. Add 100 µL of EV71 Standard and testing samples into appropriate wells. Mix gently for 10 seconds.
2. Incubate at 37°C for 30 minutes without shaking.
3. Wash the plate 5 times with Wash Buffer (1x).
4. Add 100 µL of mouse monoclonal Anti-EV71 HRP Working Conjugate Reagent (1X) to each well. Mix gently for 10 seconds.
5. Incubate at 37°C for 30 minutes without 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. Each laboratory should establish its own standard curve.

Standard Curve: 37˚C 30 min + 37˚C 30 min + RT 20 min (No shaking)

Concentration (ng/mL)	O.D. 450 nm
0	0.092
1.0	0.136
2.5	0.211
5.0	0.326
10	0.550
25	1.185
50	2.098
100	3.260

Resources

References

1. Lactoferrin inhibits enterovirus 71 infection of human embryonal rhabdomyosarcoma cells in vitro. Tzou-Yien Lin, Chishih Chu, and Cheng-Hsun Chiu. *J Infect Dis.* 2002 October 15; 186(8): 1161–1164. Published online 2002 September 16. doi: 10.1086/343809.
2. Enterovirus 71 from fatal and nonfatal cases of hand, foot and mouth disease epidemics in Malaysia, Japan and Taiwan in 1997-1998. H Shimizu, A Utama, K Yoshii, H Yoshida, T Yoneyama, M Sinniah, M A Yusof, Y Okuno, N Okabe, S R Shih, H Y Chen, G R Wang, C L Kao, K S Chang, T Miyamura, and A Hagiwara. Jpn J Infect Dis. 1999 February; 52(1): 12–15.
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.

Plate Layout

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