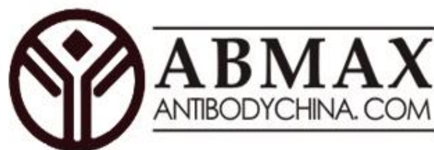

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EV71 Virus ELISA Detection Kit

Instructions for EV71 ELISA Kit (Cat# 17-0008)

1. Description

The EV71 ELISA Kit is designed for the sensitive and specific detection of EV71 virus in an antibody sandwich format. Briefly, flat-bottom 96-well plates are coated with Anti-EV71 monoclonal antibody (mAb) that captures EV71 virus in the sample. A quick wash removes any unbound virus particles. Captured EV71 is detected by a second specific Anti-EV71 mAb, which is conjugated to horseradish peroxidase (HRP). Finally, the chromogenic substrate 3,3´,5,5´-tetramethylbenzidine (TMB) is added. The amount of EV71 virus is proportional to the color generated in the coupled oxidation-reduction reaction and can be determined using a standard curve generated with known amounts of EV71.

2. Kit Components

EIA plate coated with Anti-EV71 mAb, 12 wells X 8 strips	17-0008a
Anti-EV71 Detection mAb, HRP Conjugate, 12ml	17-0008b
20X Washing Buffer, 50ml	SI00190
Sample Dilution Buffer, 12ml	SI00191
Substrate Solution A, 7ml	SI00192
Substrate Solution B, 7ml	SI00193
Stop Solution, 7ml	SI00194
Assay Protocol	

Storage Conditions: When stored at 2-8°C, the product is stable for six months. Return each component to 4°C immediately after use.

3. General Considerations

For quality control of EV71 vaccine production or in vitro research use only

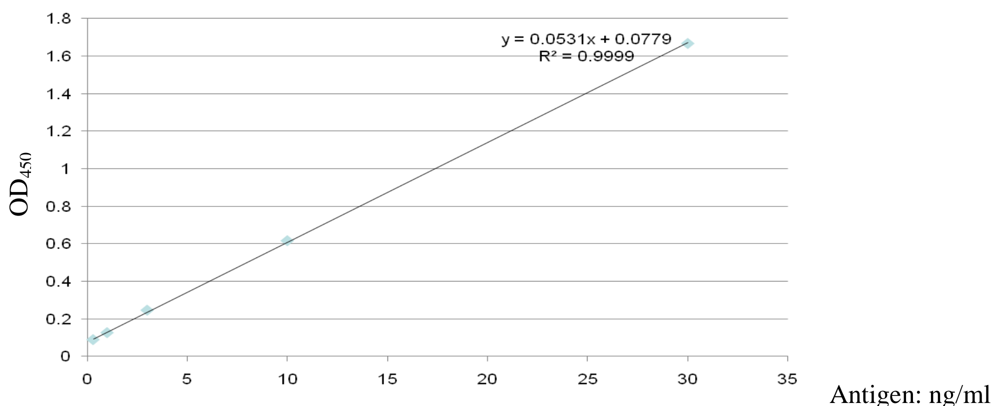
Not for in vitro diagnostic application

4. Protocol for EV71 Virus Detection

4A. Before using, pre-warm all the reagents to room temperature (18 – 25℃). Dilute 20X Washing Buffer to 1000 ml and mix well.
4B. Dilute samples with Sample Dilution Buffer and add 100μl into each well of the ELISA plate. Set negative control and incubate at 37℃ for 60 min.
4C. Remove samples from wells and wash all wells five times with prepared 1X Washing Buffer. Remove residual solution with paper pat.
4D. Add 100μl of HRP-Conjugated Anti-EV71 Detection mAb to each well. (Careful not to touch or scratch the surface of the wells). Incubate plate at 37℃ for 45 min.
4E. Remove samples from wells and wash all wells five times with prepared 1X Washing Buffer. Remove residual solution with paper pat.
4F. Add 50μl each of Substrate solution A and Substrate solution B into each well. Mix thoroughly with shaking. Incubate at 37℃ for 10~15 min. Avoid light.
4G. Stop the reaction by adding 50μl of Stop Solution.
4H. Record the absorbance at 450 nm on a plate reader within 30 min.

Cut off value=2.1 X N (N is A450 for negative control. set N=0.05, if N<0.05)
If A450 for negative control is larger than 0.15, this assay is failed.
If sample A450 is smaller than cut off value, this sample is negative. And vice versa.

Sensitivity: 1 ng/ml
Liner Range and Standard Curve: 0.3 ~ 30 ng/ml



5. Caution

5A. Avoid cross contamination.
5B. Follow reader measure as standard.
5C. All samples and buffers are added or removed with pipette.
5D. Do not mix reagents from different batches.

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