Human enterovirus 71 virus (EV71) antibody (IgM) ELISA kit

Cat#: orb406107 (ELISA Manual)

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with anti-human IgM. Samples are pipetted into the wells and any human IgM present is bound by the pre-coated anti-human IgM. After washing, EV71 antigen conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of human EV71 antibody (IgM) bound in the initial step. The color development is stopped and the intensity of the color is measured.

SPECIFICITY

This assay has high sensitivity and excellent specificity for detection of human EV71antibody (IgM). No significant cross-reactivity or interference between human EV71 antibody (IgM) and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between human EV71 antibody (IgM) and all the analogues, therefore, cross reaction may still exist.

PRECISION

Intra-assay Precision (Precision within an assay): CV%<15%
Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV%<15%
Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Reagents	Quantity
Assay plate	1(96wells)
Positive Control	1 x 0.5 ml
Negative Control	1 x 0.5 ml
HRP-conjugate	1 x 12 ml
Sample Diluent	1 x 12 ml
Wash Buffer (20 x concentrate)	1 x 50 ml
Substrate A	1 x 6 ml
Substrate B	1 x 6 ml
Stop Solution	1 x 6 ml
Adhesive Strip (For 96 wells)	4
Instruction manual	1

STORAGE

Unopened kit	Store at 2 -8°C. Do not use the kit beyond the expiration date.
Opened kit	May be stored for up to 1 month at 2 -8° C.

***Provided this is within the expiration date of the kit.**

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630nm.
- An incubator which can provide stable incubation conditions up to 37°C±0.5°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- 100mland 500mlgraduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

Serum Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 15 minutes at 1000 ×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 ×g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Note:

1. Biorbyt is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1month) or -80°C (≤2month) to avoid loss of bioactivity and contamination.
3. Grossly hemolyzed samples are not suitable for use in this assay.
4. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
5. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

REAGENT PREPARATION

Note:

- **Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.**

- Bring all reagents to room temperature (18-25°C) before use for 30min.
- Distilled water is recommended to be used to make the preparation for reagents or samples. Contaminated water or container for reagent preparation will influence the detection result.

Wash Buffer(1x)- If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 ml of Wash Buffer Concentrate (20 x) into deionized or distilled water to prepare 1000 ml of Wash Buffer (1 x).

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples be assayed in duplicate.

1. Prepare all reagents, and samples as directed in the previous sections.
2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
3. Set a Blank well without any solution.

4. Set three wells for Negative Control, one well for Positive control. Add 100µl of **Negative Control** or **Positive Control** per well.
5. Add 100µl of **Sample Diluent** to rest wells, then add 10µl of **Sample** per well.
6. Cover with the adhesive strip provided. Incubate for 50min at 37°C. A plate layout is provided to record controls and samples assayed.
7. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (200µl) using a squirt bottle, multi-channel pipette, manifold dispenser or auto washer and let it stand for 30-60 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating ordecanting. Invert the plate and blot it against clean paper towels.
8. Add 100µl of **HRP-conjugate** to each well except Blank well. Cover the microtiter plate with a new adhesive strip. Incubate for 30min at 37°C.
9. Repeat the aspiration/wash process for five times as in step 7.
10. Add 50µl of **Substrate A** and 50µl **Substrate B** to each well. Incubate for 15 minutes at 37°C. **Protect from light.**
11. Add 50µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 630 nm. Subtract readings at 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. TMB Substrate is easily contaminated. TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

CALCULATION OF RESULTS

For calculation the valence of human EV71antibody(IgM), compare the sample well with control.

(1) The average Negative Control OD values must no more than 0.10.
(2) Positive Control OD Values must no less than 0.30.
(3) The OD value of Blank well must no more than 0.08.
(4) A cutoff value was defined as the average Negative Control value plus 0.10. (If the average value of ODnegative＜0.05, calculate it as 0.05.)

- While ODsample ≥ cutoff value: Positive
- While ODsample ＜ cutoff value: Negative