Mouse rotavirus (RV) antigen (Ag) ELISA kit

Catalog Number
EK0354

This immunoassay kit allows for the in vitro semi-quantitative determination of mouse Rotavirus antigen concentrations in feces.

This instruction must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with an antibody specific to Rotavirus antigen. Standards or samples are then added to the appropriate microtiter plate wells with a HRP-conjugated antibody preparation specific for Rotavirus antigen to each microplate well and incubated. Then a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain Rotavirus antigen, HRP-conjugated antibody will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of Rotavirus antigen in the samples is then determined by comparing the O.D. of the samples to the standard curve.

MATERIALS PROVIDED

Reagent	Quantity
Assay plate	1
Positive Control	1 x 0.8 ml
Negative Control	1 x 0.8 ml
HRP-conjugate	1 x 6 ml
Wash Buffer	1 x 6 ml
Substrate A	1 x 5 ml
Substrate B	1 x 5 ml
Stop Solution	1 x 5 ml

STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.

SAMPLE STORAGE

Store samples at -20°C(four weeks)；

Store samples at 2-8°C(48 hours)；

Avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate. All the reagents should be added directly to the liquid level in the well. The pipette should avoid contacting the inner wall of the well.

1. Set three Negative Control wells, two Positive Control wells.Set one Blank well.
2. Add 50µl of **Sample**, **Positive Control** and **Negative Control** per well. Add 50µl of **HRP-conjugate** to each well, **not to Blank well.** Cover the microtiter plate with a adhesive strip. Incubate for 15 minutes at room temperature.
3. Aspirate each well and wash, Add 50µl of **Wash Buffer** to each well.
4. Wash by filling each well with **ddH_2O** (350µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, stay for 30 seconds. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining liquid by aspirating or decanting. Invert the plate and blot it against clean paper towels. **Repeating the process ten times for a total of ten washes,**
5. Add 50µl of **Substrate A** and 50µl of **Substrate B** to each well. Incubate for 10 minutes at room temperature. Keeping the plate away from drafts and other temperature fluctuations in the dark.
6. Add 50µl of **Stop Solution** to each well when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

For calculation the valence of mouse Rotavirus antigen, compare the sample well with control..

A cutoff value was defined as the average Negative Control value plus 0.1. (If the ODnegative ＜0.05, calculate as 0.05).

While OD_{sample} ≧ cutoff value: Positive

While OD_{sample} ＜ cutoff value: Negative

Positive Control OD values must no less than 0.8 , If Positive Control OD values less than 0.8, repeat the test.

Negative Control OD Values must no more than 0.1, If Negative Control OD values more than 0.1, repeat the test.

LIMITATIONS OF THE PROCEDURE

- 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.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- 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.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. 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 Substrate Solution.