Horse rotavirus ELISA kit

Catalog Number. orb339911

For the qualitative determination of horse rotavirus (RV) antigen (Ag) concentrations in feces.

This package insert must be read in its entirety before using this product.

If You Have Problems

Technical Service Contact information

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In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

PRINCIPLE OF THE ASSAY

This assay employs the qualitative enzyme immunoassay technique.
The microtiter plate provided in this kit has been pre-coated with an antibody specific to Rotavirus antigen. Samples are pipetted into the wells with antibody conjugated Horseradish Peroxidase(HRP). Any Rotavirus antigen present in samples will bind to the pre-coated specific antibody. 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 horse rotavirus (RV) antigen (Ag) 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 horse rotavirus (RV) antigen (Ag). No significant cross-reactivity or interference between horse rotavirus (RV) antigen (Ag) and analogues was observed.

Note: Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between horse rotavirus (RV) antigen (Ag) 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(96 wells)
Negative Control	1 x 800 µl
Positive Control	1 x 800 µl
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
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 two weeks 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 540 nm or 570 nm.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Absorbent paper for blotting the microtiter plate.
- Deionized or distilled water.
- Pipettes and pipette tips.

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

- **Feces** Direct collect water liquid for detection; If no fluid, dilute samples 20-30 times with normal saline. Remove particulates by centrifugation for 1-2 minutes at 1000rpm and assay immediately or aliquot and store samples at -20°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 48 hours may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤four weeks) to avoid loss of bioactivity and contamination.
3. 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 30 min.
- 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.

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 and controls 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)，three **Negative Control** wells and two **Positive Control** wells.
4. Add 50 µl of **Negative Control**, **Positive Control** or **Sample** per well; then add 50 µl of **HRP-conjugated** to each well except the **Blank** well. Cover with the adhesive strip provided. Incubate for 15 minutes at room temperature.
5. Aspirate each well and wash. Add **Wash Buffer** (50 µl) to each well.
6. Wash by filling each well with **ddH_2O**(250 µl) using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand 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 ordecanting. Invert the plate and blot it against clean paper towels. **Repeating the process ten times for a total of ten washes**.
7. Add 50 µl of **Substrate A** and 50 µl **Substrate B** to each well. Incubate for 10 minutes at room temperature. Protect from light.
8. Add 50 µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
9. Take blank well as zero, determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm.

***Samples may require dilution. Please refer to Sample Preparation section.**

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 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 liquid 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 seconds 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 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 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 horse rotavirus (RV) antigen (Ag), compare the sample well with control.

Cut-off Value= the average value of $OD_{negative}$＋0.10（if $OD_{negative}$ <0.05, calculate it as 0.05).

1. The negative control OD value should be less than 0.1, and the positive control OD value should be greater than 0.8. Or else, repeat the test.
2. Determination of results:
   - While OD_{sample}≥Cut-off Value: Positive;
   - While OD_{sample}＜Cut-off Value: Negative.
3. If OD_{sample} close to the Cut-off Value, we recommend repeating the experiment.