Human Rotavirus IgG antibody (RV-Ab-IgG) ELISA Kit

Cat #: orb1668815 (manual)

96 Tests

FOR RESEARCH USE ONLY; NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!
PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

INTENDED USE

For the qualitative detection of **Human Rotavirus IgG antibody (RV-Ab-IgG)** concentration in **solid samples (feces), liquid samples (feces).**

REAGENTS AND MATERIALS PROVIDED

Reagents	Quantity	Reagents	Quantity
Assay plate (96 Wells)	1	Instruction manual	1
Positive Control	1 x 0.5 mL	Negative Control	1 x 0.5 mL
HRP-Conjugate	1 x 6 mL	Wash Buffer	1 x 6 mL
Substrate A	1 x 6 mL	Substrate B	1 x 6 mL
Adhesive Films	4		

MATERIALS REQUIRED BUT NOT SUPPLIED

- Precision single or multi-channel pipettes and disposable tips.
- Deionized or distilled water.
- Eppendorf Tubes for serial dilution samples.
- Normal saline (NS).
- Absorbent paper for blotting the microtiter plate.

STORAGE

Unopened kit	Store at 2 - 8°C. Do not use past kit expiration date.	
Opened/Reconstituted Reagents	Coated assay plate	May be stored for up to 1 month at 2 - 8°C. Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal, and avoid the damp.
	Positive Control	May be stored for up to 1 month at 2 - 8°C.
	Negative Control	
	HRP-Conjugate	
	Wash Buffer	
	Substrate A	
	Substrate B	

***Once the standard is reconstituted it must be used immediately and cannot be stored for repeated use.**

INTRODUCTION

Rotavirus is the most common cause of severe vomiting and diarrhoea among infants and young children. It is a genus of double-stranded RNA virus in the family Reoviridae. Nearly every child in the world has been infected with rotavirus at least once by the age of five. Immunity develops with each infection, so subsequent infections are less severe; adults are rarely affected. There are eight species of this virus, referred to as A, B, C, D, E, F, G and H. Rotavirus A, the most common species, causes more than 90% of rotavirus infections in humans. The virus is transmitted by the faecal-oral route. It infects and damages the cells that line the small intestine and causes gastroenteritis (which is often called "stomach flu" despite having no relation to influenza). Although rotavirus was discovered in 1973 by Ruth Bishop and her colleagues by electron micrograph images and accounts for up to 50% of hospitalizations for severe diarrhoea in infants and children, its importance has been underestimated within the public health community, particularly in developing countries. In addition to its impact on human health, rotavirus also infects animals, and is a pathogen of livestock.

PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with Anti-RV-Ab-IgG. Samples are then added to the appropriate microtiter plate wells with a Horseradish Peroxidase (HRP)-conjugated Anti-RV-Ab-IgG and incubated. Then substrate solutions are added to each well. Only those wells that contain RV-Ab-IgG and enzyme-conjugated Anti-RV-Ab-IgG will exhibit a change in color.

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.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.

- If samples generate values higher than the highest standard, dilute the
- samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, 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 ELISA Kit, the possibility of interference cannot be excluded.

STABILITY

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% within the expiration date under appropriate storage condition.

The loss rate was determined by accelerated thermal degradation test. Keep the kit at 37ºC for 4 and 7 days, and compare O.D.values of the kit kept at 37ºC with that of at recommended temperature. (referring from China Biological Products Standard, which was calculated by the Arrhenius equation. For ELISA kit, 4 days storage at 37oC can be considered as 6 months at 2-8oC, which means 7 days at 37oC equaling 12 months at 2-8oC).

Note:

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

SAMPLE COLLECTION AND STORAGE

- **Solid samples (feces)** Take a little of the sample, put into 1 mL centrifugal tube, add 200 µL normal saline, shake properly for 2 minutes, Centrifuge and collect the supernates for assay. Samples must be free of contamination.
- **Liquid samples (feces)** The liquid sample is need diluted with deionized water at 1:3 (1 mL liquid sample + 2 mL deionized water), centrifuge and collect the supernates for assay. Samples must be free of contamination.

Note:

1. Samples to be used within 2 days may be stored at 2-8ºC, otherwise samples must be stored at -20ºC (≤ 1 month) or -80ºC (≤ 2 months) to avoid loss of bioactivity and contamination.

2. Sample hemolysis will influence the result, so hemolytic specimen cannot be detected.

3. When performing the assay, bring samples to room temperature.

SAMPLE PREPARATION

Human feces samples require no dilution before test. **The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.**

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, 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 2 - 8°C.
3. Set a Blank well without any solution. Add 50 µL of Negative Control, Positive Control and Sample per well. And add 50 µL of HRP-Conjugate to each well (**Note: Do not add to Blank!**), mix well. Cover the microtiter plate with adhesive films. Incubate for 10 minutes at 25 °C.
4. Aspirate each well, **add 50 µL of Wash Buffer**, and **wash with deionized or distilled water**, repeating the process for a total of ten washes. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 50 µL of Substrate A and 50 µL of Substrate B to each well, mix well. Incubate for 10- 15 minutes at 25 °C. Keeping the plate away from drafts and other temperature fluctuations in the dark.

***Samples may require dilution. See Sample Preparation section.**

CALCULATION OF RESULTS

For calculation the valence of human RV-Ab-IgG, compare the sample well with control.

1) Negative Control must no color.
2) Positive Control must blue color.
3) If blue color observed: Positive

If no color observed: Negative

IMPORTANT NOTE

1. The instruction manual also suits for the kit of 48T, but all reagents of 48T kit are reduced by half.
2. There may be some foggy substance in the wells when the plate is opened at the first time. It will not have any effect on the final assay results. Do not remove microtiter plate from the storage bag until needed.
3. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.
4. Samples or reagents addition: Please use the freshly prepared Standard. 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 standards and 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.

5. 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.
6. 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.
7. Controlling of reaction time: Observe the change of color after adding Substrate Solution (e.g. observation once every 10 minutes), Substrate Solution should change from colorless or light blue to gradations of blue.
8. Substrate Solution is easily contaminated. Substrate Solution should remain colorless or light blue until added to the plate. Please protect it from light.
9. Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism.
10. Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results.
11. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.
12. Limited by the current condition and scientific technology, we can't completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So there might be some qualitative and technical risks to use the kit.
13. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.
14. Kits from different batches may be a little different in detection range, sensitivity and color developing time.
15. Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.
16. Kits from different manufacturers with the same item might produce different results, since we haven’t compared our products with other manufacturers.
17. Valid period: six months.