



## **Human Rotavirus antigen(RV Ag) ELISA Kit**

**Catalog Number. CSB-E05033h**

**For the qualitative determination of human Rotavirus antigen(RV 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**

Phone: 86-27-87582341

Fax: 86-27-87196150

Email: [tech@cusabio.com](mailto:tech@cusabio.com)

Web: <https://www.cusabio.com/>

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 human Rotavirus antigen 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 Rotavirus antigen. No significant cross-reactivity or interference between human Rotavirus antigen and analogues was observed.

**Note:** Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between human Rotavirus antigen and all the analogues, therefore, cross reaction may still exist.

## **PRECISION**

### **Intra-assay Precision (Precision within an assay): CV%<15%**

Three positive samples were tested twenty times on one plate to assess.

### **Inter-assay Precision (Precision between assays): CV%<15%**

Three positive samples 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 $\mu$ l
Positive Control	1 x 800 $\mu$ l
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. CUSABIO 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. 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-conjugate** 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<sub>2</sub>O**(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 or decanting. 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.**

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 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 human Rotavirus antigen, compare the sample well with control.

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

1. The negative control OD value should be no more than 0.1, and the positive control OD value should be no less than 0.8. Or else, repeat the test.
2. Determination of results:
  - While  $OD_{\text{sample}} \geq \text{Cut-off Value}$ : Positive;
  - While  $OD_{\text{sample}} < \text{Cut-off Value}$ : Negative.
3. If  $OD_{\text{sample}}$  close to the Cut-off Value, we recommend repeating the experiment.



# 人轮状病毒抗原(RV Ag)酶联免疫试剂盒

## 使用说明书

【产品编号】CSB-E05033h

【预期应用】ELISA 法定性检测人粪便样本中轮状病毒抗原。

### 【产品性能指标】

- 1、 精密度：批内差 CV%<15%，批间差 CV%<15%
- 2、 特异性：本试剂盒特异性检测人轮状病毒抗原。

### 【实验原理】

用纯化的特异性抗体包被酶标板，制成固相载体。向微孔中先加入待测样品、辣根过氧化物酶标记的抗轮状病毒抗体进行反应，经过彻底洗涤后用底物显色。底物在过氧化物酶的催化下转化成蓝色，并在酸的作用下转化成最终的黄色。颜色的深浅和样品中的轮状病毒抗原呈正相关。用酶标仪在 450nm 波长下测定吸光度（OD 值），与阴性对照孔判断样品中轮状病毒抗原水平。

### 【试剂盒组成成分】

组份	装量
酶标板 (Assay plate)	96 孔
阴性对照(Negative Control)	1 x 800 µl/瓶
阳性对照(Positive Control)	1 x 800 µl/瓶
酶结合物 (HRP-conjugate)	1 x 6 ml/瓶
洗涤液 (Wash Buffer)	1 x 6 ml/瓶
底物溶液 A (Substrate A)	1 x 5 ml/瓶
底物溶液 B (Substrate B)	1 x 5 ml/瓶
终止液 (Stop Solution)	1 x 5 ml/瓶
板贴	4

### 【存储条件及有效期】

1. 未开封的试剂盒避光保存于2-8℃。有效期为六个月。请在试剂盒标注的有效日期内使用。
2. 酶标板打开后应置有干燥剂的铝箔袋中置于2-8℃密封防潮保存，2-8℃条件下最多可保存两周。

### 【所需试剂和器材】

标准规格酶标仪；高速离心机；干净的试管和离心管；容量瓶；

系列可调节移液器及吸头；多通道移液器；蒸馏水 等

### 【样本采集及保存】

粪便：直接吸取水样液体检测；若无液体，则用生理盐水稀释 20-30 倍，1000rpm 离心 1-2min，取上清即可立即检测；或进行分装，并将标本放于-20℃保存，但应避免反复冻融。解冻后的样品应再次离心，然后检测。

### 【注意事项】

1. 实验开始前，请提前配置好所有试剂，混匀时尽量避免起泡。
2. 用户在初次使用试剂盒时，应将各种试剂管离心数分钟，以便试剂集中到管底。

### 【操作步骤】

1. 将各种试剂移至室温（18-25℃）平衡至少 30 分钟，按前述方法配制试剂，备用。
2. 将酶标板取出，设一个空白对照孔、不加任何液体；同时设置阴性对照 3 孔、阳性对照 2 孔。
3. 各自加阴性对照、阳性对照或待测标本各 50  $\mu$ l（待测样本推荐做复孔）。
4. 每孔加入酶结合物 50  $\mu$ l（空白孔除外），充分混匀，贴上不干胶封片，置室温反应 15 分钟。
5. 洗板：弃去孔内液体，每孔加洗涤液 50  $\mu$ l，用去离子水冲洗 10 次。每次浸泡 30 秒，250  $\mu$ l/孔，甩干。
6. 每孔加底物溶液 A 和 B 各 50  $\mu$ l，振荡混匀后，室温避光显色 10 分钟，每孔加终止液 50  $\mu$ l。
7. 空白孔调零，用酶标仪在 450nm 波长依序测量各孔的光密度（OD 值）。在反应终止后 10 分钟内进行检测。

### 【操作要点】

1. 为保证检测结果的准确性，建议设双孔测定。
2. 加样时，请使用一次性的洁净吸头，避免交叉污染。加样时应尽量轻缓，避免起泡，将样品加于酶标板孔底部，切勿沿孔壁加样。
3. 为防止样品蒸发，温育过程中酶标板必须覆上板贴，实验过程中应避免酶标板处于干燥的状态。
4. 洗涤过程非常重要，不充分的洗涤易造成假阳性，在每次洗涤过程中，需要将孔内液体完全甩干，并在吸水纸上拍干，切勿将吸水纸直接放入反应孔中吸水，或用枪在孔中吸取液体。若用枪在孔中吸去液体，请勿触及板壁。
5. 一次加样时间最好控制在 10 分钟内，如标本数量多，推荐使用排枪加样。

### 【洗板方法】

手工洗板方法：吸去（不可触及板壁）或甩掉酶标板内的液体；在实验台上铺垫几层吸水纸，酶标板朝下轻拍几次；将推荐的洗板液（蒸馏水）按 250  $\mu$ l/孔注入孔内，浸泡 30 秒。根据需要，重复此过程十次。

自动洗板：如果有自动洗板机，应在熟练使用后再用到正式实验过程中。

### 【数据处理】

临界值=阴性对照 OD 值均值+0.10（阴性对照 OD 值小于 0.05 时，以 0.05 计）

1. 阳性对照 OD 值不低于 0.8 且阴性对照 OD 值不高于 0.1 时，表示该试验结果有效；否则应重新试验；
2. 结果判定：样品 OD 值 $\geq$ 临界值，表示阳性；  
样品 OD 值<临界值，表示阴性。
3. 如果样品 OD 值在临界值附近，建议重复实验。

### 【说明】

1. 本试剂盒仅供研究使用。
2. 中、英文说明书可能会有不一致之处，请以英文说明书为准。
3. 刚开启的酶标板孔中可能会含有少许水样物质，此为正常现象，不会对实验结果造成任何影响。
4. 不要用其它生产厂家的试剂替换试剂盒中的试剂。
5. 不同批号试剂不能混用。
6. 底物请避光保存。