# Rotavirus Antigen Capture ELISA

## Product Insert

**Photometric Format**

**Cat. No. RK288-2 (2 x 96 wells)**

**For Research Use Only**

### Introduction

Rotaviruses are the main cause of acute gastroenteritis, especially in children under the age of two years. Rotaviruses have been identified in almost 50% of the feces of children with gastroenteritis, responsible for 600,000-800,000 death annually. Rotavirus infections occur frequently during the winter months.

Gastroenteritis from enteric viruses can be mortal in risk populations such as children, the elderly or immunosuppressed individuals. Characteristic symptoms include vomiting, hydro-diarrhea for between 3 and 8 days, high temperature and stomach pains. Rotaviruses transferred via the fecal-oral route are eliminated in large quantities into the intestine, so that hospital-borne infections from rotaviruses are regarded very seriously, particularly in baby stations and pediatric clinics, and are difficult to control. Early and reliable detection so that rotaviruses can be recognized and further infections avoided is therefore very important.

Virusys has developed a highly sensitive and specific enzyme immunoassay for the detection of Rotavirus nucleoprotein antigen in complex sample matrices derived from both human and veterinary sources.

**Virusys Corporation**
P.O. Box 56 ● Taneytown, MD 21787
Website www.virusys.com E-mail support@virusys.com

The test kit incorporates proprietary diluents that are designed to prevent the development of nonspecific signal derived from complex sample matrix effects and/or the nonspecific adsorption of reactive test components which result in improvements in both sensitivity and specificity .

The kit is available in a standard photometric detection format (RK288-2 or RK288-5). The kit has been tested against a variety of rotavirus subtypes for sensitivity and potentially interfering viruses and bacteria for specificity.

### Components

1. Rotavirus Antigen Capture Plate (96 tests)-2 ea.
2. Sample Preparation Reagent (1x)-12 ml
3. Rotavirus Positive Control (1x)-1 ml
4. Rotavirus Negative Control (1x)- 2 x 1.5 ml
5. Wash Buffer (20x)-50 ml
6. Rotavirus Detection Antibody, Biotin-labeled (1x)-22 ml
7. Streptavidin-HRP (1x)-22 ml
8. Chromagen Solution (1x)-22 ml
9. Stop Solution (1x)-22 ml
10. Sample Dilution Tray-2 ea.

### Optional Components

RK289 Rotavirus Antigen Calibration Kit

### Storage

Store all kit components at 2-8° C. Crystal formation may occur in the wash buffer concentrate during prolonged storage at 2-8° C. The crystals can be re-dissolved by swirling the bottle in warm tap water.

### Procedure

1. Remove the kit from storage and allow it to warm to room temperature.
2. Determine the number of test wells needed. Use one well for each sample. In addition, include one well for the **Rotavirus Positive Control** and three wells for the **Rotavirus Negative Control**.
3. To begin the assay, transfer 50 µl of **Sample Preparation Reagent** to the appropriate number of wells in the dilution tray provided.
4. Add 200 µl of each sample, positive control, and negative control to the Sample Preparation Reagent. Mix by pipetting up and down several times.
5. Transfer 100 µl of sample or control to the appropriate wells of the **Rotavirus Antigen Capture Plate**.

6. Cover the plate and incubate for 30 min. at room temperature on a plate shaker set at moderate speed.
7. Add 100 µl of **Rotavirus Detection Antibody** to each well. **Do not wash the plate at this time.** Cover the plate and incubate for an additional 45 min. on a plate shaker using the same settings (Step 6).
8. Wash the wells 6x with at least 300 µl/well **1x Wash Buffer**.
9. Add 100 µl of **Streptavidin-HRP** to each well.
10. Cover the plate and incubate for 30 min. on a plate shaker using the same settings as in Step 6.
11. Wash the wells 6x with at least 300 µl/well **1x Wash Buffer**.
12. Add 100 µl of **Chromagen** to each well and incubate for 10 min. on a plate shaker.
13. Stop the reaction by the addition of 100 µl of **Stop Solution**.
14. Shake the plate for 10-15 sec. to ensure that the reaction is uniformly stopped and then read the plate in a plate reader using a 450 nm filter.

## Quality Control

1. All negative control absorbance values should be ≤0.250.
2. The positive control absorbance value should be ≥0.500.
3. The calculated value for the positive control/cut-off should be ≥2 (see below).

## Determination of Cut-off and Interpretation of Results

1. To determine the **cut-off value**, calculate the mean of the three negative control absorbance values and multiply this value by 2.
2. To interpret the results for a given sample, divide the absorbance value for the sample by the cut-off value. Calculated sample values that are >1.1 are considered reactive. Calculated sample values that are <0.9 are considered nonreactive. Calculated sample values that are ≥0.9 and ≤1.1 are considered equivocal.

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