

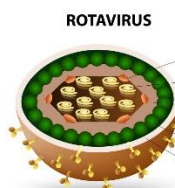
## INTENDED USE

The Human Anti-Rotavirus (EDIM) VP6 IgA ELISA Kit is an immunoassay suitable for detecting and quantifying IgA antibody activity specific for Rotavirus A VP6 capsid protein in serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use. This immunoassay is suitable for:

- Determining immune status relative to non-immune controls;
- Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization and timing;
- Qualifying and/or standardizing vaccine batches and protocols.

The kit uses recombinant proteins as antigen and there is no virus or virus derived protein in the kit. For research use only (RUO), not for diagnosis, cure or prevention of the disease.

## GENERAL INFORMATION

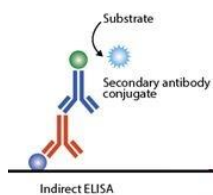


Rotavirus A (EDIM), a non-enveloped RNA virus, causes severe gastroenteritis in humans and other mammals, including mice and rats, and is readily transmitted via viral shedding in feces. Infection among infants is highly lethal; infected laboratory animals are not suitable for animal research due to compromised immunity, and modified intestinal absorption.

Human vaccines using live rotaviruses have been variable in effectiveness. Potentially safer and more efficacious vaccines not composed of live viruses include using recombinant VP6 protein, a highly immunogenic component of the intermediate capsid layer, administered either intranasally or orally along with an adjuvant (e.g., *E. coli* heat-labile toxin).

RecombiVirus™ ELISAs are 2nd generation immunoassays using purified recombinant, antigenic proteins of viruses.

## PRINCIPLE OF THE TEST



The Human Anti-Rotavirus/EDIM VP6 IgA ELISA kit is based on the binding of antibody in samples to EDIM VP6 antigen immobilized on the microwells, that is detected by anti-human IgA-specific antibody conjugated to HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color (blue), which is directly proportional

to the amount of antibody present in the sample. Stop Solution is added (converts blue to yellow color), and A450nm is then measured using an ELISA reader. The activity of antibody in samples is calculated relative to supplied calibrators.

## PRODUCT SPECIFICATIONS

### Specificity

Purified recombinant protein (*E. coli*, his-tag) of the rotavirus A (EDIM) capsid protein 6 (VP6, full length ~46 kDa) is used to coat the microwells; thus the assay is specific for antibodies directed to VP6. The Anti-Human IgA HRP conjugate reacts specifically with human IgA class antibodies; IgG, IgM and IgE antibody would not be measured above background signals.

## KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

**To Be Reconstituted:** Store as indicated.

Component	Preparation Instructions
<b>Wash Solution Concentrate (100x)</b> Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as <b>Working Wash Solution</b> and store at ambient temperature until kit is used entirely.
<b>Sample Diluent Concentrate (20x)</b> Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as <b>Working Sample Diluent</b> and store at 2-8°C until the kit lot expires or is used up.
<b>Anti-Human IgA-HRP Conjugate Concentrate (100x)</b> Part: H-HuA.112, 0.15ml	Peroxidase conjugated anti-Human IgA in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of <b>Working Sample Diluent</b> is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8°C storage.

**Ready For Use:** Store as indicated on labels.

Component	Part	Amt	Contents
<b>EDIM Ag Microwell Strip Plate</b>	300401	8-well strips (12)	Coated with recombinant EDIM VP6 antigen, and post-coated with stabilizers.
<b>Anti-EDIM Sensitivity Control</b>	300402	0.65 ml	<b>Low</b> level Anti-EDIM, in buffer with detergents and antimicrobial.
<b>Anti-EDIM Positive Control</b>	300403	0.65 ml	<b>High</b> level Anti-EDIM, in buffer with detergents and antimicrobial.
<b>Low NSB Sample Diluent</b>	TBTm	30 ml	Buffer with protein, detergents and antimicrobial. Use as is for sample dilution
<b>TMB Substrate</b>	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
<b>Stop Solution</b>	80101	12 ml	Dilute sulfuric acid.

### Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Human IgA HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
- Stock bottle to store diluted Wash Solution; 0.2 to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

## ASSAY DESIGN AND SET-UP

### Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

### Antibody Stability & Sample Dilutions

Initial dilution of serum into **Working Sample Diluent** (WSD) is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into **Low NSB Sample Diluent** (LNSD), which provides the lowest assay background, should be at least 10 times the initial dilution and performed the same day as the assay.

Example:

Initial (1/5): **10ul serum + 40ul WSD** [or 0.1ml + 0.4ml]  
Further (1/100): **10ul initial (1/5) + 190 ul LNSD**

### Assay Design

Review Calculation of Results (p5-7) and Limits of the Assay (above) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the **Sensitivity Control**. This is usually 1/100 or greater dilution for human sera with normal levels of IgG and IgM.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
- Run the **Positive** and **Sensitivity Controls**, which validate that the assay was performed to specifications: the **Positive Control** should give a high signal (>1.5 OD); the **Sensitivity Control** should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).

### Plate Set-up

Bring all reagents to room temperature (18-30° C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 4 Control wells and 2 wells for each sample and internal control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
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## Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

### 1. 1<sup>st</sup> Incubation [50 or 100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

### 2. 2<sup>nd</sup> Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Human IgA HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

### 3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

### 4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

### 5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

## PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). <http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf>

# Recombivirus™ Human Anti-Rotavirus (EDIM) VP6 IgA ELISA Kit

Cat. #. AE-300432-1, 96 tests

For detection and Quantitation of IgA  
Antibodies to VP6 Capsid Protein of  
Rotavirus A in Serum, plasma or other  
biological fluids

For in vitro research use only, not for therapeutic use.



**ALPHA DIAGNOSTIC  
INTERNATIONAL**

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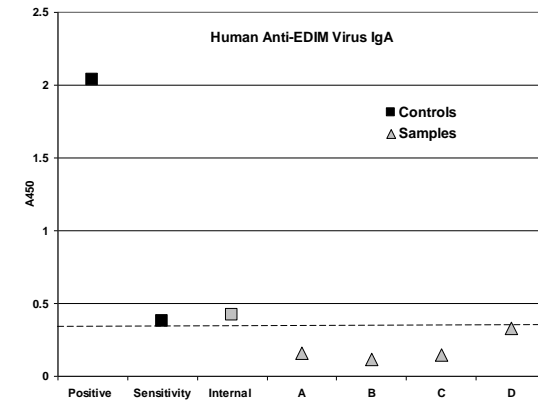
Phone (210) 561-9515 • Fax (210) 561-9544  
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Email: [service@4adi.com](mailto:service@4adi.com)  
[www.4adi.com](http://www.4adi.com)

## INTERPRETATION OF RESULTS

### A. Antibody Activity Threshold Index

Compare Samples to **Sensitivity Control** or **Internal Control**  
= **Positive/Negative Cut-off.**

#### Example:



#### Results

The **sensitivity** of the assay to detect anti-EDIM IgA, from either natural infection or immunization, is controlled so that the **Sensitivity Control** represents a threshold OD for most true positives in human serum diluted in the Low NSB Sample Diluent at 1:100 or greater. Visual inspection of the data in the above graph shows the following:

**Positive Control** – clearly positive, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.

**Sensitivity Control** – a ‘Cut-off’ line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. The is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

**Internal Control** – a true positive human serum that represents the lab’s experience in distinguishing low positive from negative samples. This should be run in each assay to supplement the Sensitivity Control for Positive/Negative discrimination purposes.

**Samples A,B,C,D** – 3 samples (A, B, C) are negative: below the threshold; 1 sample (D) is borderline: not clearly above the threshold.

The Sensitivity Control can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative, as follows:

- ❖ Divide each Sample net OD by the Sensitivity Control net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

## INTERPRETATION OF RESULTS (cont)

### B. Positive Index

Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.

This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

#### Example:

Sample	Assay Net OD		Calculated Antibody Activity	
	Control	Exptl	Control	Exptl
1	0.248	<b>P</b> 2.212	0.79	<b>7.04</b>
2	0.290	<b>S</b> 0.452	0.92	<b>1.44</b>
3	0.186	<b>I</b> 0.541	0.59	<b>1.72</b>
4	0.276	<b>U</b> 0.212	0.88	0.68
5	0.161	<b>U</b> 0.122	0.51	0.39
6	0.173	<b>M</b> 1.491	0.55	<b>4.75</b>
7	0.153	<b>M</b> 0.694	0.48	<b>2.21</b>
8	0.211	<b>N</b> 1.487	0.67	<b>4.74</b>
9	0.145	<b>N</b> 0.546	0.46	<b>1.74</b>
10	0.110	<b>U</b> 0.263	0.35	0.84
Mean	0.195			
SD	0.0595			
Mean +2 SD	<b>0.314</b>	<b>= Positive Index</b>		

#### Results

**Experimental** Samples are represented as follows:

- P** – Positive Control
- S** – Sensitivity Control
- I** – Internal Control; lab’s threshold positive serum
- U** – Uninfected human sample
- M** – samples represent injection of EDIM VP6 antigen (same as used for plate coating) into humans; positive indicates presence of the EDIM VP6 in the inoculum.
- N** – Naturally infected human samples.

## INTERPRETATION OF RESULTS (cont)

### Method C. Titers from Sample Dilution Curves

The titer of antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. The Positive and Sensitivity Control values can be used to normalize inter-assay values.

#### Calculations

1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index  
**= IgA Antibody Activity Units**

#### Limits of the Assay

- The sensitivity of the assay may be increased to perhaps convert a borderline sample to a positive by using a lower dilution of the sample, e.g., 1/50. The values of negatives may increase, so an alternative threshold should be established using known negatives to develop a **Positive Index** (page 6), or by using known **Internal Controls** as discriminator for a **Threshold Control** (instead of the kit Sensitivity Control).
- The assay detects and quantifies IgA antibodies directed to the VP6 capsid protein. It may be possible for an animal to have Rotavirus/EDIM infection without producing antibodies specific to VP6
- Anti-EDIM antibody levels of an infected or immunized individual may be below detection threshold related to the time course of the infection, e.g., too early for positive titer development.

#### Assay Sensitivity

The EDIM antigen coating level, HRP conjugate concentration, and Low NSB Sample Diluent are optimized to differentiate anti-EDIM IgA from background (non-antibody) signal with human serum samples diluted 1:100.