INTENDED USE

The **Porcine Anti-Rotavirus (EDIM) VP6 IgG** ELISA Kit is an immunoassay suitable for detecting and quantifying IgG antibody activity specific for Rotavirus A (Epizootic Diarrhea of Infant Mice: EDIM) 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

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 animal viruses. The ADI Porcine Rotavirus/EDIM VP6 IgG ELISA is designed with high sensitivity for the detection of antibodies derived from immunizations or natural EDIM infection.

PRINCIPLE OF THE TEST

The **Porcine Anti-Rotavirus/EDIM VP6 IgG** ELISA kit is based on the binding of pig anti-EDIM IgG in samples to EDIM VP6 antigen immobilized on the microwells, and anti- EDIM VP6 IgG antibody is detected by anti-pig IgG-specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti- EDIM VP6 IgG present in the sample. Stopping Solution is added to terminate the reaction and absorbance at 450nm is then measured using an ELISA microwell reader. The presence of pig IgG antibody in samples is determined relative to anti-EDIM VP6 Controls.

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-Pig IgG HRP conjugate reacts specifically with pig IgG class antibodies; IgA, IgM, and IgE antibodies would not be measured above background signals.

Assay Sensitivity
The EDIM antigen coating level, HRP conjugate concentration, and Low NSB Sample Diluent are optimized to differentiate anti-EDIM IgG from background (non-antibody) signal with pig serum samples diluted 1:200.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8ºC 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
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8ºC until the kit lot expires or is used up.
Anti-Pig IgG- HRP Conjugate Concentrate (100x) Part: H-PiG.211, 0.15ml	Peroxidase conjugated anti-Pig IgG in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8º C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
EDIM Ag Microwell Strip Plate	300401	8-well strips (12)	Coated with recombinant EDIM VP6 antigen and post-coated with stabilizers.
Anti-EDIM Sensitivity Control	300404S	0.65 ml	**Low** level Anti-EDIM in buffer with detergents and antimicrobial.
Anti-EDIM Positive Control	300404P	0.65 ml	**High** level Anti-EDIM in buffer with detergents and antimicrobial.
Low NSB Sample Diluent	TBTm	30 ml	Buffer with protein, detergents, and anti-microbial. Use as is for sample dilution
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	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-Pig IgG 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

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

Assay Design

Review Calculation of Results (p. 5-7) and Limits of the Assay 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 1:200 or greater dilution for pig 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 (18-30º 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.
- Add 200-300ul Working Wash Solution to each well and let stand for about 1-5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

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. 1st 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. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Pig IgG HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 1.

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.

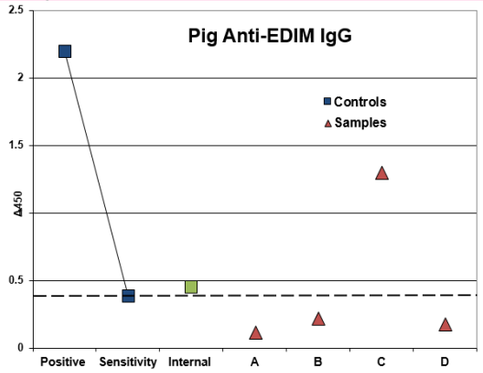
INTERPRETATION OF RESULTS

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 IgG, from either natural infection or immunization, is controlled so that the **Sensitivity Control** represents a threshold OD for most true positives in pig serum diluted in the Low NSB Sample Diluent at 1:200 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 pig serum that represents the lab's experience in distinguishing low positive from negative samples (not in kit). 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, D) are negative: below the threshold; 1 sample (C) is positive: 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:

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

Results

Experimental Samples are represented as follows:
P – Positive Control
S – Sensitivity Control
I – Internal Control; lab's threshold positive serum
U – Uninfected pig sample
M – samples represent injection of EDIM VP6 antigen (same as used for plate coating); positive indicates presence of the EDIM VP6 in the inoculum.
N – Naturally infected pig samples.

ASSAY PERFORMANCE

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

= IgG 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 IgG 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.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Controls, 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

Instruction Manual No. M-AE-300470-1

Recombivirus™ Porcine Anti-Rotavirus (EDIM) VP6 IgG

Cat. #AE-300470-1

For Quantitation of IgG Antibodies to VP6 Capsid Protein of Rotavirus A in Serum

For research use only (RUO), not for diagnosis, cure or prevention of the disease.

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ELISA Kit Components	Amount	Part
EDIM Antigen Coated Strip Plate	8-well strips (12)	300401
Anti-EDIM Sensitivity Control	0.65 ml	300404S
Anti-EDIM Positive Control	0.65 ml	300404P
Anti-Pig IgG HRP Conjugate (100X)	0.15 ml	H-PiG.211
Sample Diluent (20x)	10 ml	SD20T
Low NSB Sample Diluent	30 ml	TBTm
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-AE-300470-1