INTENDED USE

The Rat anti-Kilham Rat Virus (KRV) IgG ELISA Kit is an immunoassay suitable for detecting and quantifying IgG antibody activity specific for KRV major capsid protein (VP2) antigen in serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use.

GENERAL INFORMATION

Three serogroups of parvoviruses affect rats: PV (including **Kilham rat virus, KRV**), RPV and H-1 (Toolans). Parvoviruses are typically linear, non-segmented ssDNA viruses with an average genome size of 5Kb, and target replicating tissue causing cell and tissue destruction. This explains why RV, in its active stage, will interfere with the formation of offspring, causing small litters, stillborns, or the resorption of litters.The viral capsid of a parvovirus is made up of two or three proteins, known as VP1-3, that form an icosahedral structure; VP2 is highly immunogenic.

KRV infection may be diagnosed by ELISA, measuring rapidly rising antibody titers (8-12 days after infection) to KRV antigen. Animals infected with KRV are not suitable for animal research; in addition to lower growth rates, KRV may predispose to secondary bacterial infection, cause infertility, and death in susceptible strains. Besides infecting animals, KRV may also contaminate cell lines, transplantable tumors and other biological products; these should be tested by rat antibody production (RAP), using ELISA to detect anti- KRV after immunization.

RecombiVirus™ ELISAs are 2nd generation immunoassays using highly purified, recombinant and antigenic proteins of animal viruses. The ADI Rat Anti-KRV IgG ELISA is designed with high sensitivity for the detection of antibodies during KRV infection.

PRINCIPLE OF THE TEST

The Rat Anti-KRV IgG ELISA kit is based on the binding of anti-KRV IgG in samples to KRV antigen immobilized on the microwells, and anti- KRV IgG antibody is detected by anti- 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- KRV 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 rat IgG antibody in samples is determined relative to rat anti-KRV IgG Controls.

PRODUCT SPECIFICATIONS

Specificity
Purified recombinant protein (E.coli) of the Kilham rat virus major capsid protein (VP2, full length ~65kda, >95%) is used to coat the microwells; thus the assay is specific for antibodies directed to KRV VP2 protein. The Anti-rat IgG HRP conjugate reacts specifically with rat IgG class antibodies that bind to the KRV antigen on the plate. IgA, IgM and IgE antibody would not be measured above background signals.

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

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{0}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
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 4oC for long term and ambient temp. for short term.
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 2-8^{o}C until the kit lot expires or is used up.
Anti-Rat IgG- HRP Conjugate Concentrate (100x) Part: H-RtG-512, 0.15ml	Peroxidase conjugated anti-rat 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 2-8^{o} C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
KRV Ag Microwell Strip Plate	310111	8-well strips (12)	Coated with KRV VP2 antigen, and post-coated with stabilizers.
Rat Anti-KRV Sensitivity Control	310112	0.65 ml	**Low** level Rat Anti-KRV, in buffer with detergents and antimicrobial.
Rat Anti-KRV Positive Control	310113	0.65 ml	**High** level Rat Anti-KRV, 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.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Rat IgG HRP Concentrate.
- 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 and ELISA plate washer

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 (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 rat 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^{o} 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 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 [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-Rat IgG 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.

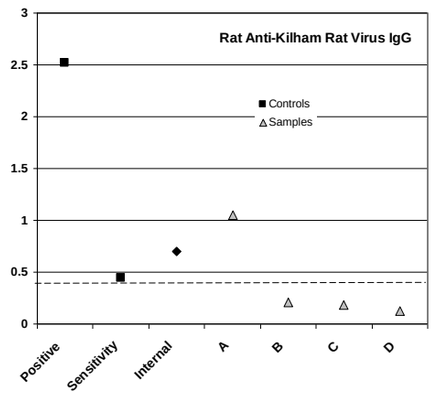
INTERPRETATION OF RESULTS

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-KRV IgG, from either natural infection or RAP (rat antibody production), is controlled so that the **Sensitivity Control** represents a threshold OD for most true positives in rat 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 from an infected animal 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 (B, C, D) are negative: below the threshold; 1 sample (A) 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.

This calculation was used to represent Assay Precision, page 7.

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	0.195	**P** 2.345	0.90	**10.86**
2	0.189	**S** 0.374	0.88	**1.73**
3	0.137	**R** 1.080	0.63	**5.00**
4	0.155	**I** 0.582	0.72	**2.69**
5	0.073	**N** 0.779	0.34	**3.61**
6	0.149	**N** 0.990	0.69	**4.58**
7	0.090	**N** 0.773	0.42	**3.58**
8	0.121	**N** 0.993	0.56	**4.60**
9	0.149	**N** 2.357	0.69	**10.91**
10	0.122	**U** 0.225	0.56	1.04
Mean	0.138			
SD	0.039			
Mean +2 SD	**0.216**	= **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 rat sample
R – Rat Antibody Production (RAP) sample represents injection of KRV antigen (same as used for plate coating) into rats; positive indicates presence of the KRV in the inoculum.
N – Naturally infected rat samples.

ASSAY PERFORMANCE

Precision

Samples and Controls (as shown in the graph, page 5) were assayed in duplicate in 5 separate runs, to provide a measure of between-assay reproducibility.

The data are represented using the value of the Sensitivity Control in each assay to calculate a **Threshold Index** for each control and samples (as described on page 5).

Sample	Ave OD	Threshold Index (mean)	Inter-assay %CV
Positive Control	2.33	7.83	**3.6**
Sensitivity Control	0.30	**1.00**	**0**
Internal Control	0.56	1.87	**4.1**
Natural Positive	0.88	2.94	**6.8**
RAP Positive	1.03	3.47	**6.4**

Results

The coefficient of variation (%CV) shows the reproducibility of the assay for measuring one antibody activity (sample or control) relative to another antibody activity (**Sensitivity Control**). Variation increases in the threshold region; for this reason, consider running additional tests for borderline samples.

Notes:

- 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)

Limits of the Assay

- The assay detects and quantifies IgG antibodies directed to the major capsid protein VP2. It may be possible for an animal to have KRV infection without producing antibodies specific to VP2.
- Anti-KRV antibody levels of an infected animal 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: http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

Instruction Manual No. M-AE-310110-1

Recombivirus™

Rat Anti-Kilham Rat Virus (KRV/VP2) IgG ELISA Kit

Cat #. AE-310110-1, 96 tests

For Quantitation of Anti-KRV-VP2 IgG in serum, plasma or other biological fluids

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

ALPHA DIAGNOSTIC INTERNATIONAL

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.

Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777
service@4adi.com
www.4adi.com

ELISA Kit Components	Amount	Part
KRV Antigen Coated Strip Plate	8-well strips (12)	310111
Rat Anti-KRV Sensitivity Control	0.65 ml	310112
Rat Anti-KRV Positive Control	0.65 ml	310113
Anti-Rat IgG HRP Conjugate (100X)	0.15 ml	H-RtG-512
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-310110-1