INTENDED USE

The **Mouse anti- Rabies Virus Glycoprotein (RVG) IgG** ELISA Kit is an immunoassay suitable for detecting and quantifying IgG antibody activity specific for RVG, in serum or plasma. Other biological fluids, including tissue culture medium, may be validated for use.

GENERAL INFORMATION

Rabies is a fatal zoonotic disease of serious public health and economic significance worldwide. The rabies virus glycoprotein (RVG) has been the major target for subunit vaccine development, since it harbors domains responsible for induction of virus-neutralizing antibodies, infectivity, and neurovirulence.

In 1984 researchers at the Wistar Institute developed a recombinant vaccine called V-RG by inserting the glycoprotein gene from rabies into a vaccinia virus. The V-RG vaccine has since been commercialized by Merial under the trademark Raboral. It is harmless to humans and has been shown to be safe for various species of animals that might accidentally encounter it in the wild. V-RG has been successfully used in the field in Belgium, France, Germany and the United States to prevent outbreaks of rabies in wildlife. The vaccine is stable under relatively high temperatures and can be delivered orally, making mass vaccination of wildlife possible by putting it in baits. RVG has since been expressed in various expression systems, and in DNA constructs, aiming for improved vaccines.

ADI has cloned, expressed, and purified the full length glycoprotein for use in developing ELISAs to measure antibodies against RVG in various species. These kits are designed for studying efficacy of existing vaccines and preparations of more effective HPV vaccine formulations.

PRINCIPLE OF THE TEST

The Mouse Anti-RVG IgG ELISA kit is based on the binding of anti-RVG IgG in samples to RVG antigen immobilized on the microwells, and anti- RVG 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- RVG 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 mouse IgG antibody in samples is determined relative to anti-RVG Calibrators.

PRODUCT SPECIFICATIONS

Specificity
Purified recombinant (his tag; E.coli) RVG protein (protein accession #CAU03682.1, 501-aa) is used to coat the microwells; thus, no other antibody specificity is detectable in the assay. HPVL1s from HPV6, 11, 16, and 18 subtypes share ~50% sequence homology. The Anti-mouse IgG HRP conjugate reacts specifically with mouse IgG class antibodies; IgA, IgM and IgE antibody would not be measured above background signals.

Assay Sensitivity
The RVG antigen coating level and HRP conjugate concentration are optimized to differentiate anti-RVG IgG from background (non-antibody) signal with mouse serum or plasma samples diluted 1:100.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{O}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 2-8ºC until the kit lot expires or is used up.
Anti-Mouse IgG-HRP Conjugate Concentrate (100x) Part: H-MsG.2a11, 0.15ml	Peroxidase conjugated anti-mouse 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
RVG Antigen Coated Strip Plate	600-121	8-well strips (12)	Coated with recombinant RVG protein, and post-coated with stabilizers.
Anti-RVG Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	600122B 600122C 600122D 600122E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-RVG; in buffer with antimicrobial as stabilizers.
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-Mouse 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 & Dilution
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 (1/50): **10**ul initial (1/5) + **90**ul LNSD (1/50)

Assay Design
Review Interpretation of Results and Limits of the Assay (p5-7) 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 **1 U/ml Calibrator**. This is usually 1/100 or greater dilution for mouse serum/plasma 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 a set of **Calibrators**, which validate that the assay was performed to specifications: **10 U/ml** should give a high signal (>1.5 OD); **1 U/ml** 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 8 Calibrator wells and 2 wells for each sample 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-Mouse 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.

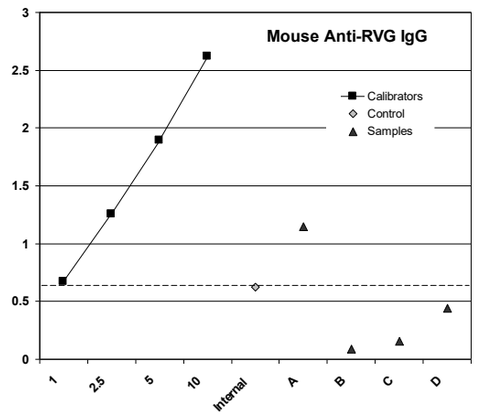
INTERPRETATION OF RESULTS

A. Antibody Activity Threshold Index

Compare Samples to **1 U/ml Calibrator** or **Internal Control**

= Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti-RVG IgG, from either natural infection or vaccination, is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in mouse serum diluted to 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of an anti-RVG antibody, derived from RVG vaccination, shows the OD range of the assay; high value indicates optimal sensitivity of the assay.
1 U/ml: 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 1 U/ml Calibrator 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 1 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

- ❖ Divide each Sample net OD by the 1 U/ml Calibrator 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	0.325	2.281 **C**	0.75	**5.29**
2	0.272	1.581 **C**	0.63	**3.67**
3	0.133	0.998 **C**	0.31	**2.32**
4	0.194	0.453 **C**	0.45	**1.05**
5	0.289	0.767 **P**	0.67	**1.78**
6	0.319	0.982 **E**	0.74	**2.28**
7	0.332	0.401 **I**	0.77	0.93
8	0.291	0.351 **E**	0.68	0.81
9	0.402	0.325 **E**	0.93	0.75
10	0.253	0.16 **E**	0.59	0.37
Mean	0.281			
SD	0.075			
Mean +2 SD	**0.431**	= **Positive Index**		

Results

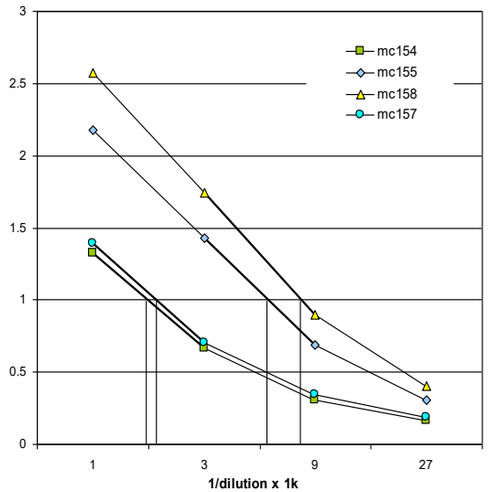
Experimental Samples are represented as follows:

C – Calibrator
P – Positive Control
I – Internal Control; lab's threshold positive serum
E – Experimental sample

ASSAY PERFORMANCE

C. Antibody Titer
The most accurate method for comparing antibody potencies is by calculation of a titer, using an OD reading midrange in the dilution curves of each antibody as **Index.** In the example below, **IgG** titers were calculated as inverse of the dilution that produced a **1.0 OD** in the assay.

Example:



Titer Values
mc154 = 1.72 kU
mc155 = 5.70 kU
mc157 = 1.85 kU
mc158 = 7.90 kU

Limits of the Assay

- The assay detects and quantifies IgG antibodies directed to the RVG protein. Animals vaccinated or infected with the rabies virus may not produce antibodies specific to RVG.
- Anti-RVG antibody levels of an infected or vaccinated animal may be below detection threshold related to the time course of the occurrence, e.g., too early for positive titer development.

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: http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

Instruction Manual No. M-600-130-MRG

Mouse Anti-RVG (Rabies Virus Glycoprotein) IgG ELISA Kit

600-130-MRG

For the Detection and Quantitation of Anti-RVG IgG in Mouse Serum/Plasma

For research use only, not for diagnostic or therapeutic use.

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ELISA Kit Components	Amount	Part
RVG Antigen Coated Strip Plate	8-well strips (12)	600-121
Anti-RVG Calibrator 1 U/ml	0.65 ml	600-122B
Anti-RVG Calibrator 2.5 U/ml	0.65 ml	600-122C
Anti-RVG Calibrator 5 U/ml	0.65 ml	600-122D
Anti-RVG Calibrator 10 U/ml	0.65 ml	600-122E
Anti-Mouse IgG HRP Conjugate (100X)	0.15 ml	H-MsG.2a11
Sample Diluent (20x)	10 ml	SD20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	M-600-130-MRG