INTENDED USE

The **Mouse Anti-Rabies IgG** ELISA Kit detects and quantifies rabies virus -specific IgG in mouse serum or plasma of vaccinated, immunized and/or infected hosts. 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 standardizing vaccine batches & protocols.

GENERAL INFORMATION

Rabies is a viral disease (Lyssavirus; ssRNA) that causes acute encephalitis (inflammation of the brain) in warm-blooded animals. It is transmitted by animals, most commonly by a bite from an infected animal, and is almost invariably fatal if post-exposure prophylaxis is not administered prior to the onset of severe symptoms.

Humans and animals have been protected, and the disease eradicated in certain geographical regions, by vaccination. Most vaccines have used whole inactivated virus that has been grown in a variety of cell types; vaccines using recombinant proteins of the rabies virus are also available. For rabies control of wildlife, vaccines in bait have proven effective. Improvement of the efficacy of vaccines is an active area of investigation.

The ADI anti-Rabies ELISA is designed with high sensitivity for discriminating lower level antibodies, with specially formulated diluents to minimize interfering background signals.

PRINCIPLE OF THE TEST

The **Mouse Anti-Rabies IgG** ELISA kit is based on the binding of mouse anti- rabies in samples to rabies antigen immobilized on the microwells, and anti- rabies IgG antibody is detected by anti-mouse 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-rabies 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 activity of mouse antibody in samples is determined relative to anti-rabies calibrators.

PRODUCT SPECIFICATIONS

Specificity
Antigens prepared from whole-inactivated rabies virus subtypes 1-3 are used to coat the microwells; stabilizing postcoat contains BSA; thus, no other antibody specificity is detectable in the assay. The anti-Mouse IgG HRP conjugate primarily detects IgG, and does not react with IgM, IgA or IgE class antibodies above background.

Assay Sensitivity
The rabies-coated plate, anti-Mouse IgG HRP concentration, and Low NSB Sample Diluent are optimized to differentiate anti-rabies IgG from background (non-antibody) signal with mouse serum samples diluted 1:100.

Calibrator Values
The Calibrators are dilutions of anti-rabies antibody. Values are assigned as arbitrary anti-rabies virus activity units (see Limits of the Assay).

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 refrigerated for long term and at lab temperature 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-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 2-8^{o}C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Rabies Antigen Coated Plate	600-051	8-well strips (12)	Coated with rabies antigen, and post-coated with stabilizers.
Anti-Rabies Calibrators			
10 U/ml 25 U/ml 50 U/ml 100 U/ml	600-042B 600-042C 600-042D 600-042E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-rabies antibody in arbitrary activity Units; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
Anti-Rabies Positive Control	600-042PC	0.65 ml	Serum with anti-rabies reactivity; [Value range on label]
Low NSB Sample Diluent	TBTm **Not** for HRP dilution	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-Mouse IgG HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
- Stock bottle to store diluted Wash Solution; 200ml 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. 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 5 times the initial dilution and performed the same week 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 (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 **10 U/ml Calibrator**. This is usually 1:100 or greater dilution for mouse serum 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 **Anti-Rabies Positive Control**; the value range is on the label.
- Run a set of **Calibrators**, which validate that the assay was performed to specifications: **100 U/ml** should give a high signal (>1.5 OD); **10 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 (18-30^{o} 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.

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

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 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

Calculation of Results
Consider several data reduction methods to best represent the relationships among experimental and control groups, to determine **Positive Immune** and **Negative Non-immune**, and to **Quantitate** positive antibody levels.

Method A. Antibody Activity [ELISA Signal & Sample Dilution]
Represent data as net OD units (A450 signal; blank subtracted) ÷ dilution = **Total Activity Units**.

A Calibrator value in the mid-OD range (e.g., 25 U/ml) can be used to normalize inter-assay values.

Method 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 **quantifies** the positive Antibody Activity level.

Example:

	Assay Net OD		Calculated Antibody Activity	
Sample	Control	Exptl	Control	Exptl
1	0.243	2.358	0.49	**4.79**
2	0.351	0.597	0.71	**1.21**
3	0.286	1.421	0.58	**2.89**
4	0.357	1.268	0.73	**2.58**
5	0.512	0.857	**1.04**	**1.74**
6	0.342	1.296	0.70	**2.63**
7	0.298	0.608	0.61	**1.24**
8	0.285	0.369	0.58	0.75
9	0.157	0.864	0.32	**1.76**
10	0.187	0.543	0.38	**1.10**
Mean	0.302			
SD	0.095			
Mean +2 SD	**0.492**			

= **Positive Index**

CALCULATION OF RESULTS (continued)

Method B. Use of a Calibrator Curve

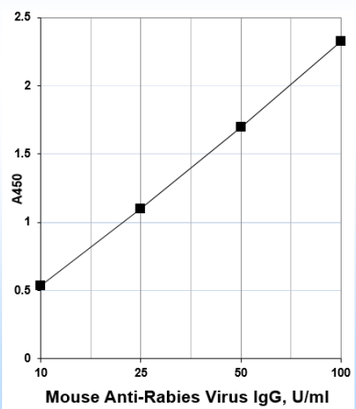
When the dilution curves of samples are parallel to the Calibrator curve (see Limits of the Assay), the anti-rabies activity units may be determined by interpolation from the Calibrator curve, as follows:

1. The results may be calculated using any immunoassay software package. If software is not available, anti-rabies activity concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples.
3. On graph paper plot the mean OD of the calibrators (y-axis) against the concentration (U/ml) of anti-rabies (x-axis). Draw the best fit curve through these points to construct the calibrator curve. A point-to-point construction is most common and reliable.
4. The anti-rabies activity concentrations in unknown samples and controls can be determined by interpolation from the calibrator curve.
5. Multiply the values obtained for the samples by the dilution factor of each sample.
6. Samples producing signals higher than the 100 U/ml calibrator should be further diluted and re-assayed.

Typical Results:

Wells	Calibrators		A450 nm
A1,2	**Sample Diluent Blank**		0.13
B1,2	10 U/ml	**Calibrator**	0.54
C1,2	25 U/ml	**Calibrator**	1.10
D1,2	50 U/ml	**Calibrator**	1.70
E1,2	100 U/ml	**Calibrator**	2.33
F1,2	Sample	**1:100**	1.23

Positive Control: = **39** U/ml



CALCULATION OF RESULTS (continued)

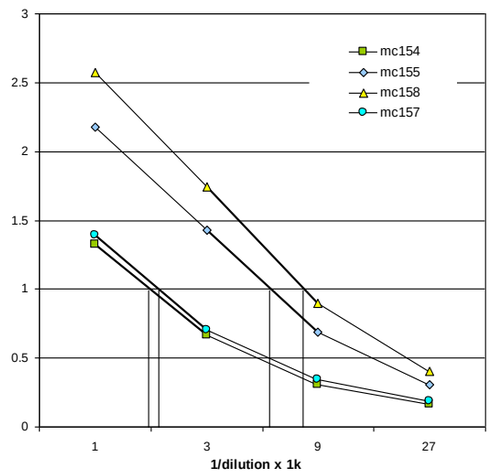
Method D. Titers from Sample Dilution Curves
The titer of antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

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. A Calibrator value in the mid-OD range (e.g., 25 U/ml) 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
   = **Total IgG Antibody Activity Units**

Example:

II. A **1.0 OD** Index was used to determine titer of 4 antibodies.



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

Instruction Manual No. M-600-030-MRG

Mouse Anti-Rabies IgG

ELISA Kit Cat. No. 600-030-MRG

For Quantitation of Anti-Rabies Virus IgG in Serum

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ELISA Kit Components	Amount	Part
Rabies Antigen Coated Microwell Strip Plate	8-well strips (12)	600-051
Anti-Rabies Positive Control	0.65 ml	600-042PC
Anti-Rabies Calibrator 10 U/ml	0.65 ml	600-042B
Anti-Rabies Calibrator 25 U/ml	0.65 ml	600-042C
Anti-Rabies Calibrator 50 U/ml	0.65 ml	600-042D
Anti-Rabies Calibrator 100 U/ml	0.65 ml	600-042E
Anti-Mouse IgG HRP Conjugate (100X)	0.15 ml	H-MsG.2a11
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-600-030-MRG