Version 12-04/24

User's Manual

G. Pig Anti-Rabies Virus IgG ELISA Kit

REF **DEIA-RV2310-14**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Guinea Pig Anti-Rabies virus IgG ELISA Kit detects and quantifies rabies virus-specific IgG in serum or plasma of vaccinated, immunized and/or infected hosts. This immunoassay is suitable for:

1. Determining immune status relative to non-immune controls;
2. Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization and timing;
3. Qualifying and standardizing vaccine batches & protocols. This kit is for research use only (RUO) and not for diagnosis cure or prevention of the disease.

General Description

Rabies is a fatal zoonotic disease of serious public health. The rabies virus is a member of the Lyssavirus genus, which have helical symmetry. The lipoprotein envelope carries knob-like spikes composed of Glycoprotein G (VRG). Beneath the envelope is the membrane or matrix (M) protein layer. The core of the virion consists of helically arranged ribonucleoprotein (RV-NP). Old vaccines are made from whole inactivated virus. New recombinant subunit vaccines are based upon purified proteins (RV-NP or VRG) that invoke strong antibodies against the rabies virus. The V-RG vaccine (Raboral/Merial) is harmless to humans and safe for various species of domestic or wild animals.

Principles of Testing

The G. Pig Anti-Rabies IgG ELISA kit is based on the binding of antibodies in samples to rabies antigens immobilized on the microwells, and bound antibody is detected by anti-G. Pig IgGspecific antibody-HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color (blue) is developed, which is directly proportional to the amount of antirabies IgG present in the sample. Stopping Solution is added to terminate the reaction (converts blue to yellow), and A450nm is then measured using an ELISA reader. The activity of G. Pig antibody in samples is determined relative to anti-rabies calibrators.

Reagents And Materials Provided

1. Rabies antigens coated Strip Plate, 8-well strips (12). Coated with rabies antigen, and post-coated with stabilizers.

2. Anti-Rabies Calibrators, Four (4) vials, each containing anti-rabies antibody in arbitrary activity Units; diluted in buffer with protein, detergents and antimicrobial as stabilizers.

10 U/ml, 0.65 ml

25 U/ml, 0.65 ml

50 U/ml, 0.65 ml

100 U/ml, 0.65 ml

3. G. Pig Anti-Rabies IgG Positive Control, 0.65 ml. G. Pig anti-rabies antiserum. Net OD > 0.5 OD

4. Low NSB Sample Diluent (LNSD), Reduces nonspecific binding, 30 ml. Buffer with protein, detergents and antimicrobial as stabilizers. Use as is for sample dilution.

5. TMB Substrate, 12 ml. Chromogenic substrate for HRP containing TMB and peroxide.

6. Stop Solution, 12 ml. Dilute sulfuric acid.

7. Wash Solution Concentrate (100×), 10ml.

8. Sample Diluent Concentrate (20×), 10ml

9. Anti-G. Pig IgG-HRP Conjugate Concentrate (100×), 0.15ml

Materials Required But Not Supplied

1. Pipettors and pipettes that deliver 100ul and 1-10ml. A multichannel pipettor is recommended.
2. Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-G. Pig IgG HRP Concentrate.
3. Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
4. Stock bottle to store diluted Wash Solution; 200ml to 1L.
5. Distilled or deionized water to dilute reagent concentrates.
6. Microwell plate reader at 450 nm wavelength.

Storage

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.

Specimen Collection And Preparation

Sample Collection and Handling

For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature Always wear gloves when handling serum-containing samples, including the standards and controls, and dispose of these samples and containers as biohazard waste.

Sample Dilution and 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): 10ul serum + 40ul WSD [or 0.1ml + 0.4ml]

Further (1/50): 10ul initial (1/5) + 90ul LNSD (1/50)

Assay Design

Review Calculation of Results and Limits of the Assay before proceeding:

1. 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 <0.5 OD. This is usually 1/100 or greater dilution for G. Pig sera with normal levels of IgG and IgM.

2. 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 (See Method A).
3. Run a set of Calibrators. Calibrators validate that the assay was performed to specifications; results can be used to normalize between-assay variation for enhanced precision. Reading values off a Calibrator curve, Method B, has limitations. See Limits of the Assay.
4. Run the G. Pig Anti-Rabies IgG Positive Control; >0.5 net OD.
5. Run a range of sample dilutions for expected higher positives that allows calculation of antibody Titer (when specific titer is at least 4-fold higher than non-immune). See Method C.
6. Run samples in duplicate if used for quantitation; non-immunes that are significantly lower than immunes may be run in singlicate. The Calibrators that are used for quantitation, e.g., for between-assay normalization, should be run in duplicate. When determining titer from a dilution curve, singlicates can be run if more than two dilution points are used for titer calculations.

Plate Preparation

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

1. Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample and control to be assayed.
2. Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
3. 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.

Reagent Preparation

1. Wash Solution Concentrate (100×)

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.

2. Sample Diluent Concentrate (20×)

Dilute 0.5ml + 9.5ml with distilled or deionized water as needed for HRP Conjugate and Sample Dilution. Label as Working Sample Diluent and store at 2-8°C until the kit lot expires or is used up.

3. Anti-G. Pig IgG - HRP Conjugate Concentrate (100×)

Peroxidase conjugated anti-G. Pig IgG in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample Diluent (WSD) is sufficient for 1 8-well strip. Use within the working day and discard. Return 100× to 2-8°C storage.

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 predetermined 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-G. 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.

Calculation

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 nonimmunepanel 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**		

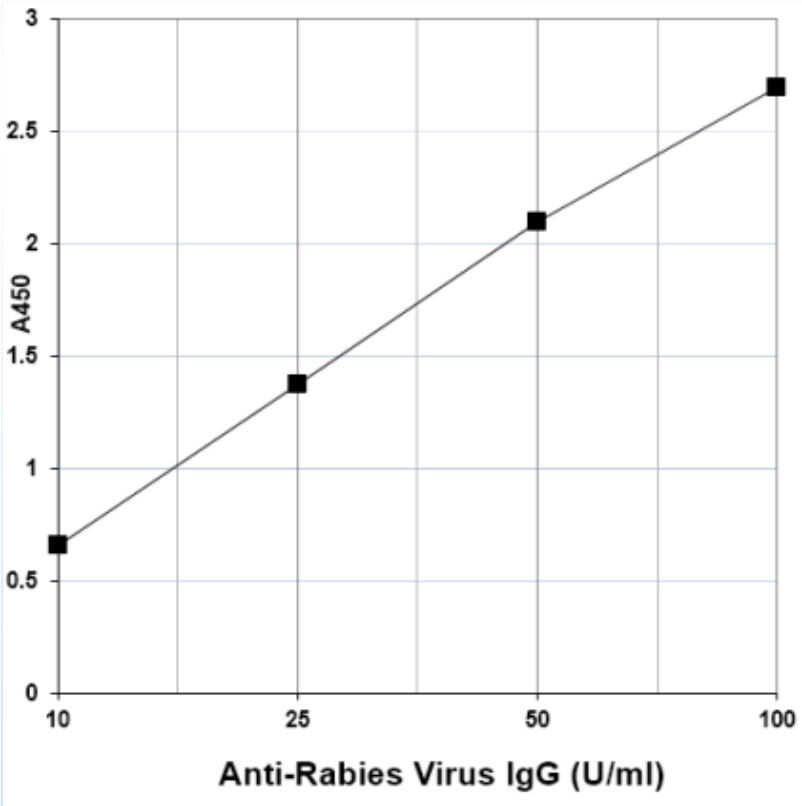
Method B. Use of a Calibrator Curve

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.

Wells	Calibrators		A450 nm
A1,2	**Sample Diluent Blank**		0.13
B1,2	10 U/ml	**Calibrator**	0.66
C1,2	25 U/ml	**Calibrator**	1.38
D1,2	50 U/ml	**Calibrator**	2.20
E1,2	100 U/ml	**Calibrator**	2.60
F1,2	Sample	**1:100**	1.21

Sample Result: **20.1** U/ml x 100 dilution = **2010** U/ml



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:

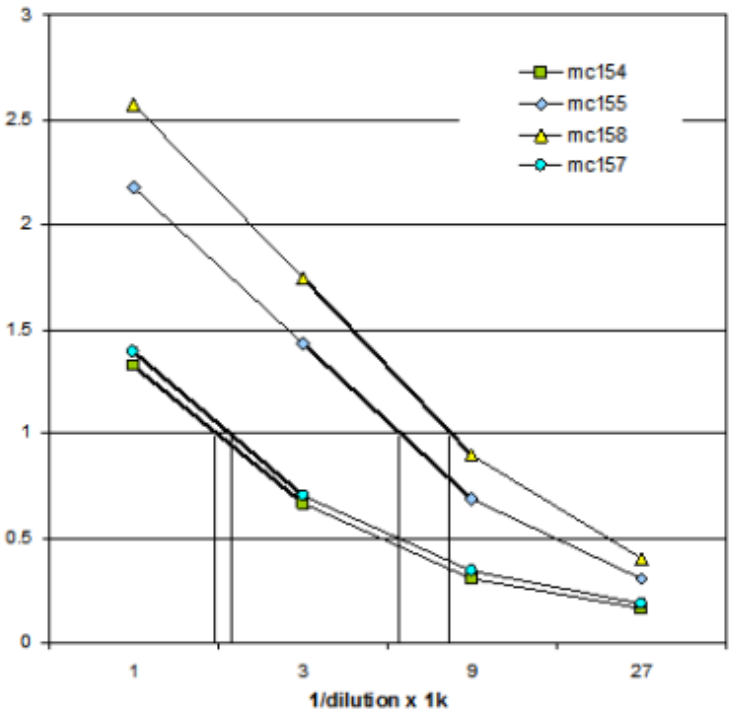
1. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals 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. Od1



Titer Values

mc154 = 1.72 kU mc155 = 5.70 kU

mc157 = 1.85 kU mc158 = 7.90 kU

Evaluation

Rabies in G. Pigs

Any warm-blooded mammal can carry or contract rabies, but the primary carriers in North America are raccoons, skunks, bats, foxes, and coyotes. Thanks to an increase in pet vaccinations, wildlife now account for more than 90 percent of all reported rabies cases. Lab grown G. pigs are not expected to have rabies antibodies. ELISA testing of few normal g. pigs sera at 1:100 show A450 values of <0.500 (background levels).

Performance Characteristics

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).

Sensitivity

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

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-G. Pig IgG HRP conjugate primarily detects IgG, and does not react with IgM, IgA or IgE class antibodies above background.

Precautions

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.

Limitations

Calibrator Curve Quantitation

To quantitate antibody activity from a calibrator curve (such as provided with the kit), the dilution curve of the samples must be parallel to the calibrator curve, to avoid different values being obtained from different regions of the curve. Antibodies that are not matched in rabies avidity will often have non-parallel dilution curves. In these cases, antibody activity is best expressed as a titer relative to a reference positive such as the 5 U/ml Calibrator, or another Calibrator in the kit (see Calculation of Results).

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