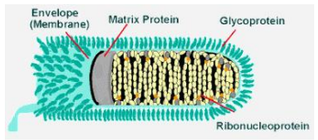
INTENDED USE

The **Human anti-Rabies Virus NP (Nucleoprotein) IgG** ELISA Kit is an immunoassay suitable for quantifying IgG antibody activity specific for Rabies NP in serum or plasma.
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

Reagents contain no virus or viral antigens. These kits are designed for studying efficacy of existing vaccines and preparations of more effective rabies vaccine formulations.The assay is for research use only (RUO).

GENERAL INFORMATION



Rabies is a fatal zoonotic disease of serious public health concern. 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.

PRINCIPLE OF THE TEST

The Human Anti-Rabies NP IgG ELISA kit is based on the binding of anti-Rabies NP IgG in samples to Rabies NP antigen immobilized on the microwells; anti- Rabies NP 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- Rabies NP IgG present in the sample. Stop Solution is added to terminate the reaction and A450nm is then measured using an ELISA reader. The presence of human IgG antibody in samples is determined relative to anti-Rabies NP Calibrators.

PRODUCT SPECIFICATIONS

Specificity

Purified recombinant (his tag; HEK293, >95%) Rabies Nucleoprotein (NP) (rabies virus/strain CVS-11) is used to coat the microwells; thus, no other antibody specificity is detectable in the assay. The Anti-human IgG HRP conjugate reacts specifically with human IgG class antibodies; IgA, IgM, and IgE antibody would not be measured above background signals.

Assay Sensitivity

The Rabies NP antigen coating level, Low NSB diluent, and HRP conjugate concentration are optimized to differentiate anti-Rabies NP IgG from background (non-antibody) signal with human serum or plasma samples diluted 1:100.

KIT CONTENTS

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. 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 4°C 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°C until the kit lot expires or is used up.
Anti-Human IgG-HRP Conjugate Concentrate (100x) Part: H-HuG.2a11, 0.15ml	Peroxidase conjugated anti-human 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°C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Rabies NP Antigen Coated Strip Plate	600-221	8-well strips (12)	Coated with recombinant Rabies NP protein and post-coated with stabilizers.
Anti-Rabies NP Calibrators			
1 U/ml 3 U/ml 8 U/ml 20 U/ml	600242B 600242C 600242D 600242E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-Rabies NP; in buffer with antimicrobial as stabilizers.
Anti-Rabies NP Positive Control	600-242-PC	0.65 ml	Serum with anti-Rabies NP reactivity; [Value range on label]
Low NSB Sample Diluent Reduces background	TBTm **Not** for HRP Conjugate dilution.	30 ml	Use as is for sample dilution. See **Assay Design**, page 3.
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-Human 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 (1/50): **10**ul initial (1/5)+ **90**ul LNSD (1/50)

Assay Design

Review Interpretation of Results and Limits of the Assay (p. 5-7) 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 human 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 the Anti-Rabies NP **Positive Control**; value range is on the label
- 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 (18-30°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 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 [100ul – 60 min; 4 washes]

- Add 100ul of sample diluent (blank), 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-Human 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.

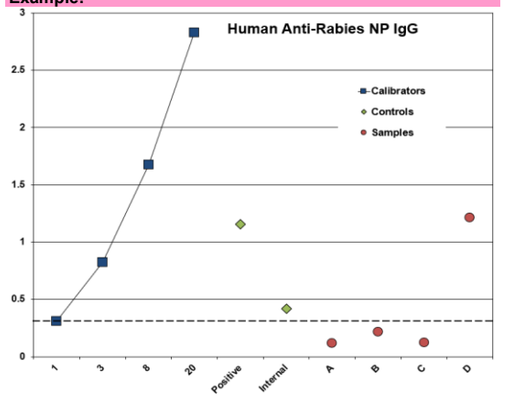
INTERPRETATION OF RESULTS

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-Rabies NP 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 human 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-Rabies NP antibody, derived from Rabies NP 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.

Positive Control – serum showing reactivity to Rabies NP; the value range is on the label. This Control may be used to gauge precision and to normalize between-assay variation.

Internal Control – a true positive from an immune human that represents the investigator's experience in distinguishing low positive from negative samples (not in kit). 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 (A, B, & C) are negative: below the threshold; 1 sample (D) 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.

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

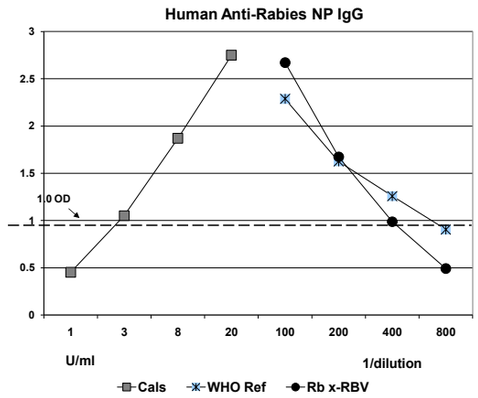
Experimental Samples are represented as follows:

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.



Results

Calibrators: The Calibrator titer value can be used to normalize between-assay sample titer values. Titer: **2.8 U/ml**

WHO International Reference (15 IU/ml): human immunoglobulin containing antibodies to rabies virus (NIBSC, code RAI). Titer: **660 –** [15 IU/ml ÷ 660] = **22.8** mIU/ml.

Rb x-RBV: antibody from rabbit immunized with whole rabies virus vaccine (Imrab 3; Merial). Titer: **395**

Calibrator Curve Quantitation

To quantitate antibody activity from a calibrator curve (such as provided with the kit, or the WHO Std), 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. In these cases, antibody activity is best expressed as a titer relative to a reference positive, as shown above.

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.

Human Anti-Rabies NP IgG ELISA Kit

Cat. #600-220-HRV

For the Detection and Quantitation of Anti-Rabies NP (Nucleoprotein N) IgG in Human Serum/Plasma/Biological Samples

This kit is for research use only (RUO), not for therapeutic use.

ALPHA DIAGNOSTIC INTERNATIONAL

4638 N Loop 1604 W • San Antonio • Texas 78249 • USA
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777 Email: service@4adi.com

ELISA Kit Components	Amount	Part
Rabies NP Antigen Coated Strip Plate	8-well strips (12)	600-221
Anti-Rabies NP Positive Control	0.65 ml	600-242PC
Anti-Rabies NP Calibrator 1 U/ml	0.65 ml	600-242B
Anti-Rabies NP Calibrator 3 U/ml	0.65 ml	600-242C
Anti-Rabies NP Calibrator 8 U/ml	0.65 ml	600-242D
Anti-Rabies NP Calibrator 20 U/ml	0.65 ml	600-242E
Anti-Human IgG HRP Conjugate (100X)	0.15 ml	H-HuG.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-600-220-HRV