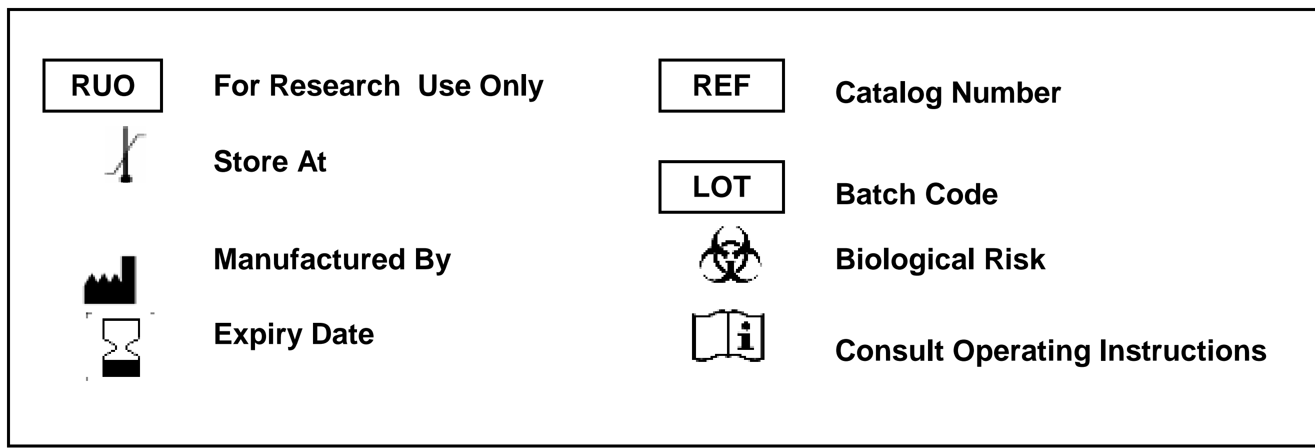
GENLISA™ Human Anti-Rabies Virus Glycoprotein IgG ELISA

REF : KBVH377

Ver 1.0

RUO

Enzyme Immunoassay for the Quantitative Determination of IgG antibody to Rabies Virus glycoprotein in human serum



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KRISHGEN BioSystems For US/Europe Customers: toll free +1(888)-970-0827 | tel +1(562)-568-5005
For Asia/India Customers: +91(22)-49198700
email: sales1@krishgen.com | http://www.krishgen.biz / www.krishgenbio.com

Introduction:

Rapid and accurate laboratory diagnosis of rabies in humans is essential for timely administration of post exposure prophylaxis. Within a few hours, a diagnostic laboratory can determine whether or not a person has been infected with rabies.

Intended Use:

Human Anti-Rabies Virus Glycoprotein IgG ELISA is specifically designed for the accurate quantitation of antibodies that have strong affinity to epitope (antigenic site) to Rabies Glycoprotein in human serum. It has proven to be an effective assay for detection of rabies.

Principle:

Microtiter wells coated with Rabies Virus are exposed to test specimens, which may contain Rabies IgG reactive antibodies. After an incubation period, unbound components in the test sample are washed away. Specific bound Rabies Glycoprotein IgG complex reacts with anti-human IgG conjugated with HRP during a second incubation period. Following a second wash cycle, specific bound enzyme conjugate is detected by reaction with the substrate solution, TMB. The assay is measured for absorbance to indicate the level of Anti-Rabies Virus glycoprotein IgG present in a sample. The assay only screens for the subtypes and is not able to distinguish between them.

Materials Provided:

1. Recombinant Rabies Virus Glycoprotein Microtiter Coated Plate (96 wells) - 1 no
2. Anti-Rabies Glycoprotein IgG Calibrator (conc) - 0.5 ml
3. Anti-Human IgG - HRP Conjugate, 12 ml
4. (20X) Wash Buffer - 60 ml
5. Sample Diluent - 30 ml
6. TMB Substrate - 12 ml
7. Stop Solution - 5 ml
8. Instruction Manual

Materials Not Provided:

1. Micro-plate Reader able to measure absorbance at 450nm.
2. Adjustable pipettes to measure volumes ranging from 50 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Lin-Lin graph paper or software for data analysis.
6. Tubes to prepare standard/sample dilutions.
7. Timer.
8. Absorbent paper.

Storage Information:

1. Store main kit components at recommended temperature indicated on individual component labels.
2. All the reagent and wash solutions are stable until the expiration date of the kit.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
4. The Substrate is light sensitive and should be protected from the direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.

2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x *g*. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles.

Reagent Preparation (all reagents should be diluted immediately prior to use):

1. Bring all reagents to Room Temperature prior to use.
2. To make Wash Buffer (1X), add 30ml of Wash Buffer (20X) in 270ml of DI water.
3. Positive Calibrator:
4. Please check the accompanying certificate for the dilution of the positive control.

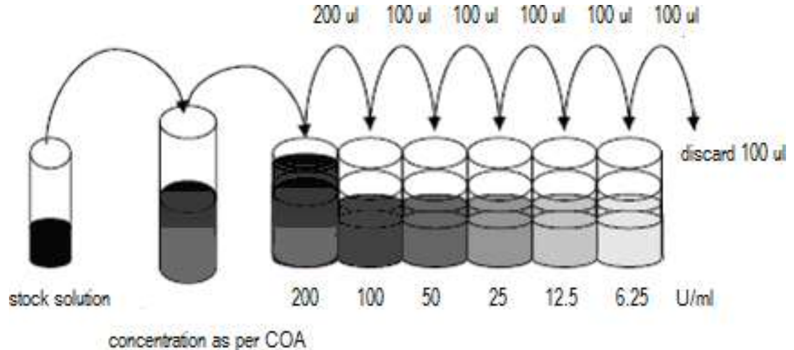
Procedural Notes:

1. For good assay reproducibility and sensitivity, proper washing of the ELISA plate to remove excess/unbound reagents is essential.
2. If the concentration of undiluted samples is less than the diluted samples, this may be indicative of the Hook Effect. High Dose Hook Effect may be observed in the samples with a very high concentration. To overcome Hook Effect sample to be assayed should be sufficiently diluted with our recommended diluent.
3. Avoid samples containing sodium azide (NaN_3), as it could destroy HRP activity of the conjugate resulting in under estimation if results.
4. All samples and Standards should be assayed at least in duplicate.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
6. The plates should be read within 15 minutes after adding the Stop Solution.
7. Make a work list in order to indentify the location of Standards and Samples.

Assay Procedure:

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. Add **100ul/well** of **Standards** and **Samples** to the plate. Perform six two-fold serial dilutions of the 200 U/ml top standard, either within the plate or in separate tubes. Thus, the standard concentrations are 200 U/ml, 100 U/ml, 50 U/ml, 25 U/ml, 12.5 U/ml and 6.25 U/ml. Sample Diluent serves as the zero Standard (0 U/ml). Seal plate and incubate at room temperature for 60 minutes.



3. Aspirate and wash plate 4 times with **Wash Buffer (1X)** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

4. Add **100ul** of **Anti-Human IgG:HRP Conjugate** solution to each well, seal plate and incubate at room temperature for 30 minutes.

5. Wash plate 4 times with Wash Buffer (1X) as in step 3. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

6. Add **100ul** of **TMB Substrate** solution and incubate in the dark for 15 minutes.

7. Stop reaction by adding **25ul** of **Stop Solution** to each well. Positive wells should turn from blue to yellow.

8. Read absorbance at 450 nm within 15 minutes of stopping reaction.

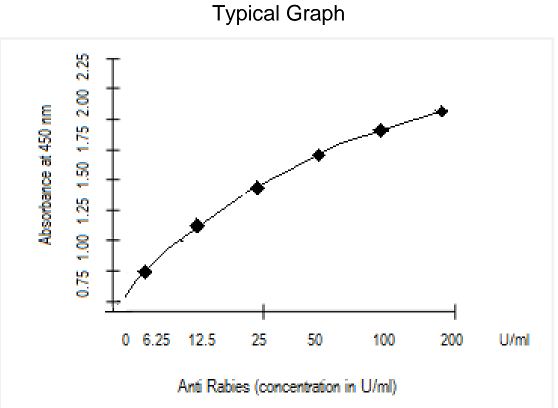
Calculation of Results:

Determine the mean absorbance for each set of duplicate or triplicate standards and samples. Plot the optical densities of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit straight line through the standard points. To determine the unknown concentrations, find the unknowns mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples were diluted, multiply by the appropriate dilution factor. Computer based curve-fitting software may be preferred.

Typical Data:

This standard curve was generated at KRISHGEN for demonstration purposes only. A standard curve must be run with each assay.

Typical Graph



Precautions:

Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.

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