INTENDED USE

The **Camel Anti-Rift Valley Fever Virus Glycoprotein 2 (RVFV-G2) IgG** ELISA Kit is an immunoassay suitable for quantifying IgG antibody activity specific for RVFV G2 in 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

The assay is for research use only (RUO) and is not intended nor validated for diagnosing Rift Valley Fever virus disease. Reagents contain no virus or viral antigens.

GENERAL INFORMATION

Rift Valley Fever virus (RVFV) is a mosquito borne zoonotic disease endemic to Africa. RVFV is a negative stranded RNA virus which belongs to the Bunyaviridae family and Phlebovirus genus. RVFV is characterized by high rates of abortion in ruminants and hemorrhagic fever, encephalitis, and blindness in humans. The RVFV genome encodes 7 proteins; the nucleoprotein (N), M, NSs, NSm, Gn, Gc, and the L protein.

Sheep, goats, camels, and cattle are the most susceptible animals affected by RVFV. Humans can become infected by RVFV through contact with aerosols of body fluids from infected animals or from the bites of mosquitoes. RVFV is classified as a category A priority pathogen by the NIH and an overlap select agent by the HHS and USDA. Currently a live-attenuated MP-12 vaccine generated from the pathogenic wild-type strain ZH548 is conditionally licensed for use for veterinary purposes in the United States. RVFV is one of several viruses identified by WHO as a likely cause of future epidemics in a new plan developed after the 2014 Ebola epidemic for urgent research and development toward new diagnostic tests, vaccines, and medicines. Current diagnosis of RVFV relies on viral isolation from tissues or serological testing using an ELISA.

PRINCIPLE OF THE TEST

The **Anti-RVFV IgG ELISA** kits are based on the binding of antibodies (IgG) in samples to the recombinant, purified RVFV antigen immobilized on the microwells. Bound antibody is detected by anti-camelid IgG-HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color is developed by the HRP substrate, which is directly proportional to the amount of anti-RVFV IgG present in the sample. Stop Solution is added to terminate the reaction, and absorbance is then measured using an ELISA reader at 450nm. The presence of antibody (IgG) in samples is determined relative to anti-RVFV Calibrators.

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 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/Conjugate Diluent** and store at 2-8o C until the kit lot expires or is used up.
Anti-Camelid IgG-HRP Conjugate Concentrate (100x) Part: H-LaG.211, 0.15ml	Peroxidase conjugated anti-camelid IgG in buffer with detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample/Conjugate Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8oC storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
RVFV G2 Coated Strip Plate	404301	8-well strips (12)	Coated with purified recombinant RVFV G2, and post-coated with stabilizers.
Anti-RVFV-G2 Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	404332B 404332C 404332D 404332E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-RVFV G2; in buffer with antimicrobial as stabilizers.
Anti-RVFV-G2 Positive Control	404332-PC	0.65 ml	Antiserum with anti-RVFV G2 reactivity; [Value range is on the label]
Low NSB Sample Diluent (LNSD)	TBTm Reduces non-specific binding	30 ml	Buffer with protein, detergents and antimicrobial. 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-Camelid 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 1:200 or greater dilution for camel serum/plasma with normal levels of IgG and IgM.
- Run the **Anti-RVFV-G2 Positive Control**; value range is on the label.
- 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 (18-30^{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.

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-Camelid 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.

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

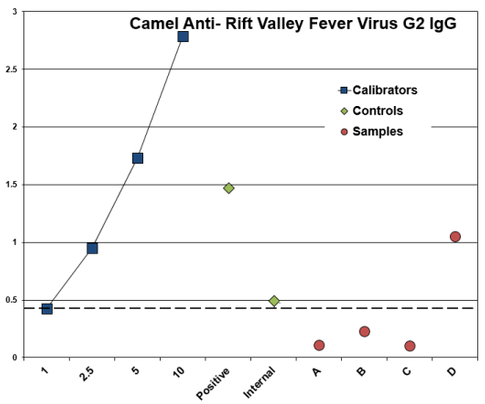
INTERPRETATION OF RESULTS

Method 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-RVFV G2 IgG, from either natural exposure or vaccination, is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in camel serum diluted to 1:200 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of an anti-RVFV G2 antibody, derived from G2 immunization, 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**. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – antiserum reactive to RVFV G2; value range is on the label. This Control can be used to assess reproducibility and/or normalize between-assay variation.

Internal Control – a true positive from an immune camel 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.

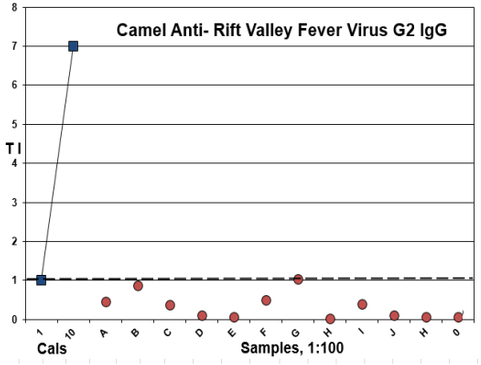
The **1 U/ml** Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative (see p6):

- 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 is **Negative** for antibody.

ASSAY PERFORMANCE

Example:

Camel Serum IgG
A panel of sera from non-immunized camelids was tested for anti-RVFV G2 IgG (1:100 dilution in Low NSB Sample Diluent). **Threshold Index** was calculated using the **1** U/ml **Cal**.



Results

Anti-RVFV G2 IgG: ten samples (10were negative (below the 1.0 threshold); two samples (B, G) were borderline at 1:100 dilution.

Notes:

1. **Positives** may be due to prior encounter with the virus or non-RVFV proteins with common epitopes, or may be an aspect of the innate immune repertoire.
2. When the **Positive Index** is **above 5.0**, using a dilution curve to calculate titer is a more accurate quantitation method (see Method C).
3. The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1:200) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1:50) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative threshold should be considered using known negatives to develop a **Positive Index (**see below) or use an **Internal Control** (Page 5).

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.

INTERPRETATION OF RESULTS (cont)

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 sera dilution.

This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

Method C. Titers from Sample Dilution Curves
The titer of elevated 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. The Positive and Sensitivity Control values 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

= IgG Antibody Activity Units

PRODUCT SPECIFICATIONS

RVFV G2 Protein

RVFV G2 protein was expressed in E. coli as his-tag fusion protein. The protein is truncated from the helical and cytoplasmic domains. RVFG G2 has 59% homology with Gabek Forest virus glycoprotein, 57% Karimabad virus glycoprotein, 55% Uriurana virus glycoprotein, and 53% Punta Toro virus M protein.

Antibody Specificity

The anti-camelid IgG-HRP used in the kit is specific for camel IgG with no significant detection of IgM, IgA, IgE or other antibodies. This kit is optimized for camel samples.

Instruction Manual No. M-RV-404350-1

Recombivirus™ Camel Anti-Rift Valley Fever Virus Glycoprotein 2 (G2) IgG ELISA Kit

Catalog # RV-404350-1, 96 tests

For the Detection and Quantitation of Anti-RVFV G2 IgG in Serum or Plasma

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

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ELISA Kit Components	Amount	Part
RVFV-G2 Coated Strip Plate	8-well strip (12)	404301
Anti- RVFV-G2 Positive Control	0.65 ml	404332PC
Anti- RVFV-G2 Calibrator 1 U/ml	0.65 ml	404332B
Anti- RVFV-G2 Calibrator 2.5 U/ml	0.65 ml	404332C
Anti- RVFV-G2 Calibrator 5 U/ml	0.65 ml	404332D
Anti- RVFV-G2 Calibrator 10 U/ml	0.65 ml	404332E
Anti-Camelid IgG HRP Conjugate (100X)	0.15 ml	H-LaG.211
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	RV-404350-1