## INTENDED USE

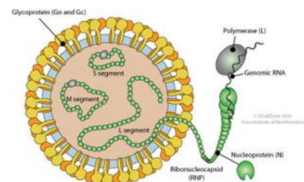
The Bovine Anti-Crimean-Congo Hemorrhagic Fever Virus (**CCHFV**) IgG ELISA Kit detects and quantifies CCHFV -specific IgG in bovine serum or plasma of vaccinated, immunized and/or infected hosts. This immunoassay is suitable for:

- 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.

This kit does not contain any CCHFV virus or virus-derived proteins. It uses highly purified recombinant protein as antigen. This kit is for research use only (RUO), not for diagnostic use.

## GENERAL INFORMATION

Crimean-Congo hemorrhagic fever (CCHF) is a widespread tick-borne viral disease, a zoonosis of domestic animals and wild animals, which may affect humans. The pathogenic virus, especially common in East and West Africa, is a member of the Bunyaviridae family of RNA viruses. Clinical disease is rare in infected mammals, but is severe, with a 30% mortality rate. Outbreaks of illness are usually attributable to handling infected animals or humans.




The virus genome is circular, ambi-sense RNA in three parts – Small (S), Middle (M) and Large (L). The L segment encodes the RNA polymerase; the M segment encodes the envelope proteins (Gc and Gn); and the S segment encodes the nucleocapsid protein. The genome is circular, ambisense RNA in three parts - Small (S), Middle (M) and Large (L). The causative organism is found in Asia, Eastern Europe, and the Middle East, a belt across central Africa and South Africa and Madagascar. The main environmental reservoir for the virus is small mammals (particularly European hare, Middle-African hedgehogs and multi-mammate rats). Ticks carry the virus to domestic animal stock. Sheep, goats and cattle develop high titers of virus in blood, but tend not to fall ill. Birds are generally resistant with the exception of ostriches. No approved vaccines available. ADI has cloned, expressed and purified CCHFV nucleoprotein (~55 kDa) that is being used as a candidate for newer subunit vaccine for CCHF. It is also used in this kit to detect antibodies to CCHFV.

## PRINCIPLE OF THE TEST

The Bovine Anti-CCHFV NP IgG ELISA kit is based on the binding of bovine anti-CCHFV IgG in samples to CCHFV NP antigen coated on the plate, and bound antibody is detected by anti-bovine IgG-specific HRP conjugate. 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- CCHFV NP IgG present in the sample. Stopping Solution is added to terminate the reaction, and A450nm is then measured using an ELISA reader. The presence of bovine IgG antibody in samples is determined relative to anti-CCHFV NP Controls.

## PRODUCT SPECIFICATIONS

### Specificity

Purified recombinant (his tag; E.coli) CCHFV nucleoprotein (NP) is used to coat the microwells; thus, no other antibody specificity is detectable in the assay. The anti-Bovine IgG HRP conjugate specifically detects IgG, with no reaction with IgM, IgA or IgE class antibodies.

### Assay Sensitivity

The CCHFV antigen coating level and HRP conjugate concentration are optimized to differentiate anti-CCHFV IgG from background (non-antibody) signal with bovine serum samples diluted 1:100.



### Calibrator Values

The Calibrators are dilutions of anti-CCHFV antibody. Values are assigned as arbitrary anti-CCHFV activity units.

## KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8ºC 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)**<br>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 ambient temperature until kit is used entirely. |
| **Sample Diluent Concentrate (20x)**<br>Cat. No. SD-20T, 10ml | Dilute 0.5ml + 9.5ml with distilled or deionized water as needed for HRP Conjugate and Sample Dilution. Label as **Working Sample/Conjugate Diluent** and store at 2-8ºC until the kit lot expires or is used up. |
| **Anti-Bovine IgG - HRP Conjugate Concentrate (100x)**<br>Part No. H-BvG.211, 0.15ml | Peroxidase conjugated anti-Bovine IgG in buffer with protein, 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 |
|---|---|---|---|
| **CCHFV Microwell Strip Plate** | 320421 | 8-well strips (12) | Coated with recombinant CCHFV antigen, and post-coated with stabilizers. |
| **Anti-CCHFV Calibrators** | | | |
| 1 U/ml<br>2.5 U/ml<br>5 U/ml<br>10 U/ml | 320442B<br>320442C<br>320442D<br>320442E | 0.65 ml<br>0.65 ml<br>0.65 ml<br>0.65 ml | Four (4) vials, each containing anti-CCHFV levels in arbitrary activity Units; diluted in buffer with protein, detergents and antimicrobial as stabilizers. |
| **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-Bovine 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

For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, including **tissue culture media**, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent.

### Assay Design

Review Calculation 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 <0.5 OD. This is usually 1:200 or greater dilution for bovine sera 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 (**See Methods A&B)**.
- 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 has limitations. See **Method C**.
- 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 D.**
- 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 Set-up

Bring all reagents to room temperature (18-30º 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 and 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 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-Bovine 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.

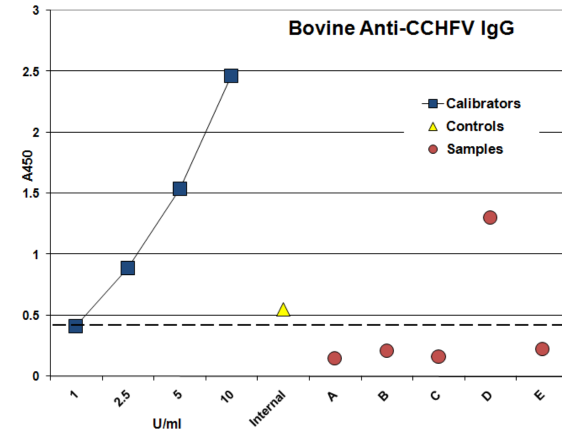
## INTERPRETATION OF RESULTS

**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-CCHFV 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 bovine 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-HPV antibody, derived from CCHFV 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**. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

**Internal Control** – a low level positive from an immunized animal that represents the lab's experience in distinguishing low positive from negative samples. This should be run in each assay to supplement the 1 U/ml Calibrator for Positive/Negative discrimination purposes.

**Samples A,B,C,D,E** – 4 samples (A, B, C, E) 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(continued)

**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 is **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:**

| Sample | Assay Net OD | | Calculated Antibody Activity | |
|---|---|---|---|---|
| | Control | Exptl | Control | Exptl |
| 1 | 0.244 | **C** 2.293 | 0.57 | **5.34** |
| 2 | 0.204 | **C** 1.490 | 0.48 | **3.47** |
| 3 | 0.237 | **C** 0.833 | 0.55 | **1.94** |
| 4 | 0.26 | **C** 0.326 | 0.61 | 0.76 |
| 5 | 0.388 | **P** 1.106 | 0.90 | **2.58** |
| 6 | 0.407 | **I** 0.310 | 0.95 | 0.72 |
| 7 | 0.288 | **E** 0.672 | 0.67 | **1.56** |
| 8 | 0.263 | **E** 0.363 | 0.61 | 0.85 |
| 9 | 0.322 | **E** 0.560 | 0.75 | **1.31** |
| 10 | 0.343 | **E** 0.490 | 0.80 | 1.14 |
| Mean | 0.295 | | | |
| SD | 0.067 | | | |
| Mean +2 SD | **0.429** | **= 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



## INTERPRETATION OF RESULTS(continued)

**Method C. 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. 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**

**Limits of the Assay**

- The assay detects and quantifies IgG antibodies directed to the NP protein. Animals may be exposed to the virus without producing antibodies specific to NP.

- Anti-CCHFV antibody levels of an immunized animal may be below detection threshold related to the time course of the infection, e.g., too early for positive titer development.

- The sensitivity of the assay may be increased to perhaps convert a borderline sample to a positive by using a lower dilution of the sample, e.g., 1/50. The values of negatives may increase, so an alternative threshold should be established using known negatives to develop a **Positive Index** (page 6), or by using known **Internal Controls** as discriminator for a **Threshold Control** (instead of the kit 1 U/ml Calibrator Control)

## PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, Positive Control, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains 1% 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



Instruction Manual No.M-AE-320450-1

# Bovine Anti-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) IgG ELISA

## Catalog #AE-320450-1, 96 tests

### For Quantitation of Anti-CCHFV IgG In Bovine Serum or Plasma or other biological fluids

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

ALPHA DIAGNOSTIC
INTERNATIONAL

**India Contact**:

LIFE TECH
Life Technologies™
Your Molecular & Cell Technology Partner

**Life Technologies (India) Pvt. Ltd.**
306, Aggarwal City Mall, Opposite M2K Pitampura,
Delhi – 110034 (INDIA).
Ph: +91-11-42208000, 42208111, 42208222
Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@lifetechindia.com
Web: www.lifetechindia.com