INTENDED USE

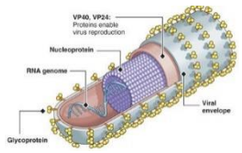
The **Human Anti-Tai Forest Virus Glycoprotein IgG** ELISA Kit is an indirect ELISA suitable for quantifying IgG antibody activity specific for the Tai Forest Ebolavirus glycoprotein in serum or plasma of vaccinated, immunized and/or infected hosts.

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 Ebola virus disease (EVD). Reagents contain no virus or viral antigens.

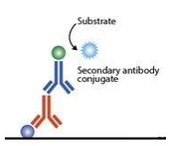
GENERAL INFORMATION



Ebola virus (**EBOV**) causes severe disease in humans and in nonhuman primates in the form of viral hemorrhagic fever. Ebola Zaire attacks every organ and tissue in the human body except skeletal muscle and bone. Strains of Ebola include: Zaire, Sudan, Reston and **Tai Forest**. All cause illness in sub-human primates. Ebola Reston has not caused illness in humans. The mortality rate of Ebola victims is between 60-90%.

Ebola virions consist of seven structural proteins.. At the center is the helical ribonucleocapsid, which consists of the genomic RNA wrapped around a polymer of nucleoproteins (**NP**). Associated with the ribonucleoprotein is the RNA-dependent RNA polymerase (L) with the polymerase cofactor (VP35) and a transcription activator (VP30). The ribonucleoprotein is embedded in a matrix, formed by the major (**VP40**) and minor (VP24) matrix proteins. These particles are surrounded by a lipid membrane derived from the host cell membrane. The membrane anchors a glycoprotein (**GP1,2**) that projects 7 to 10 nm spikes away from its surface. While nearly identical to Marburg virions in structure, Ebola virions are antigenically distinct. Viral proteins (NP, GP, and VP40) are highly immunogenic and could individually or together constitute effective vaccines. Promising vaccine candidates include DNA vaccines or those based on adenoviruses, vesicular stomatitis Indiana virus (VSIV) or filovirus-like particles (VLPs) as all of these candidates could protect nonhuman primates from Ebola virus-induced disease.

PRINCIPLE OF THE TEST



The **Anti-Tai Forest GP** IgG/IgM ELISA kits are based on the binding of antibodies (IgG/IgM) in samples to the recombinant, purified Tai Forest GP antigen (GP, NP or VP40) immobilized on the microwells. Bound antibody is detected by anti-human IgG or IgM-HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color is developed by the HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color (blue), which is directly proportional to the amount of antibody present in the sample. Stop Solution is added (converts blue to yellow color), and A450nm is then measured using an ELISA reader. The activity of antibody in samples is calculated relative to supplied 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 4^{o}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/Conjugate Diluent** and store at 2-8^{o}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/Conjugate Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8^{o}C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Tai Forest GP Coated Strip Plate	325621	8-well strips (12)	Coated with purified recombinant Tai Forest GP, and post-coated with stabilizers.
Anti-Tai Forest GP Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	325622B 325622C 325622D 325622E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-Tai Forest GP; in buffer with antimicrobial as stabilizers.
Anti-Tai Forest GP Positive Control	325622-PC	0.65 ml	Serum with anti-Tai Forest GP reactivity; [Value range is on label]
Low NSB Sample Diluent Reduces non-specific binding	TBTm **Not** for HRP Conjugate dilution.	30 ml	Buffer with protein, detergents and antimicrobial as stabilizers. 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. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Caution: Human serum and other bodily fluids may contain infectious material. Always wear gloves when handling human samples, including the standards and controls (which have been tested non-reactive for HbsAg and Anti-HIV), and dispose of these samples and containers as biohazard waste.

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 (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 mouse serum with normal levels of IgG and IgM.
- Run the Anti-Tai Forest GP Positive Control; value range is on the vial 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-Human 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: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf

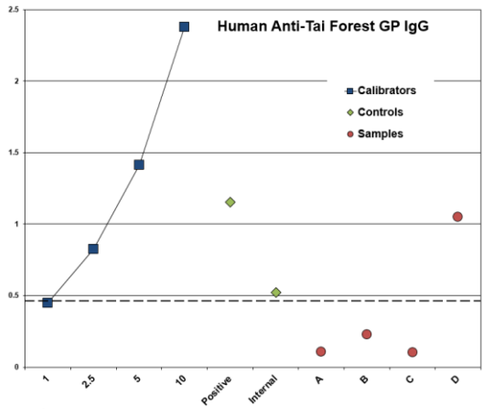
INTERPRETATION OF RESULTS

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-GP 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 1:200 or greater. Visual inspection of the data in the above graph shows the following:

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

Positive Control – antiserum with reactivity to Tai Forest GP; value range is on the vial label. The Control can be used to gauge reproducibility 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.

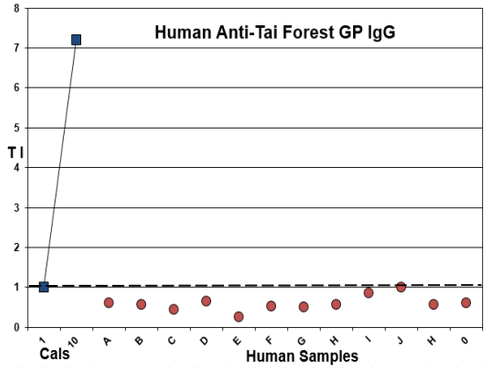
The **1 U/ml** Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative (see p6):

- 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 are **Negative** for antibody.

ASSAY PERFORMANCE

Example:

Human Serum IgG
A panel of non-immunized human sera was tested for anti-Tai Forest GP IgG (1:100 dilution in Low NSB Sample Diluent). **Threshold Index** was calculated using the **1** U/ml **Cal**.



Results

Anti-Tai Forest GP IgG: most samples were negative (below 1.0 threshold) at 1:100 dilution; 2 samples (I,J) were borderline positive.

Notes:

1. **Positives** may be due to prior encounter with the virus or non-Ebola 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 sample 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

Specificity

Tai Forest Glycoprotein (GP) coating antigen: Tai Forest Ebola Virus glycoprotein was expressed in E. coli cells as his-tag fusion protein (33-650 aa, >95%, ~70 KDa).

Sequence Conservation in Ebola subtypes.

	Zaire	Bundi-bugyo	Tai Forest	Res-ton	Sudan	Marb-urg
NP	100%	75%	69%	69%	67%	53%
VP40	100%	82%	69%	74%	75%	34%
GP	100%	65%	64%	57%	54%	NA

Although the Ebola Glycoproteins (GP) from Zaire, Sudan, Bundibugyo, Reston and the related Marburg viruses are significantly conserved (54-65%), it is not known if the antibodies elicited by one Ebola species, either from natural exposure or by vaccination, will be cross-reactive with antigens from other species. As such, the Tai Forest Ebola GP used in this kit may not react well with antibodies elicited by the antigens from other species, but this has not been experimentally confirmed.

Instruction Manual No. M-AE-325620-1

Recombivirus™ Human Anti-Tai Forest Virus Glycoprotein IgG ELISA Kit

Cat # AE-325620-1, 96 Tests

For the Detection of Anti-Tai Forest GP IgG in Serum or Plasma or other biological fluids

For research use only (RUO), not for diagnosis, cure or prevention of the disease.

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ELISA Kit Components	Amount	Part
Tai Forest GP Coated Strip Plate	8-well strips (12)	325621
Anti- Tai Forest GP Positive Control	0.65 ml	325622PC
Anti- Tai Forest GP Calibrator 1 U/ml	0.65 ml	325622B
Anti- Tai Forest GP Calibrator 2.5 U/ml	0.65 ml	325622C
Anti- Tai Forest GP Calibrator 5 U/ml	0.65 ml	325622D
Anti- Tai Forest GP Calibrator 10 U/ml	0.65 ml	325622E
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	AE-325620