INTENDED USE

The **Human Anti-HIV-2 gp36 IgM** ELISA Kit detects and quantifies IgM antibodies directed to glycoprotein **36** of human immunodeficiency virus (HIV) in human 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/or standardizing vaccine batches and protocols

The kit has no live or killed virus or viral antigens. For research use only (RUO); not for diagnosis, cure, or prevention of the disease.

GENERAL INFORMATION

Human immunodeficiency virus (**HIV**) is a lentivirus (retrovirus family) that causes acquired immunodeficiency syndrome (**AIDS**), a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive.

HIV-2 is roughly spherical with a diameter of about 120 nm, and composed of two copies of positive-sense, single-stranded RNA that codes for the virus's nine genes (gag, pol, and env, tat, rev, nef, vif, vpr, and vpx). The single-stranded RNA is tightly bound to nucleocapsid proteins and enzymes needed for the development of the virion such as reverse transcriptase, proteases, ribonuclease, and integrase.

A matrix composed of the viral protein p16 surrounds the capsid ensuring the integrity of the HIV-2 virion. This is, in turn, surrounded by the viral envelope that is composed of two layers of phospholipids taken from the membrane of the host cell when a newly formed virus particle buds from the cell. Embedded in the viral envelope are proteins from the host cell and about 70 copies of a complex HIV-2 protein that protrudes through the surface of the virus particle. This protein complex, known as **Env**, consists of a cap made of three molecules called **glycoprotein (gp) 105** and a stem consisting of **three gp36** molecules that anchor the structure into the viral envelope. This glycoprotein complex enables the virus to attach to and fuse with target cells to initiate the infectious cycle. Both these surface proteins have been considered as targets for future vaccine development.

PRINCIPLE OF THE TEST

The **Human Anti-HIV-2 gp36 IgM** ELISA kit is based on the binding of human anti-HIV-2 gp36 in samples to HIV-2 gp36 immobilized on the microwells. Anti- HIV-2 gp36 IgM antibody is detected by anti-human IgM-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-HIV-2 gp36 IgM present in the sample. Stopping Solution is added to terminate the reaction and absorbance at 450nm is then measured using an ELISA microwell reader. The activity of human antibody in samples is determined relative to anti-HIV-2 gp36 Calibrators.

PRODUCT SPECIFICATIONS

Specificity

Purified recombinant HIV-2 gp36 (E. coli expression) is used to coat the microwells. Stabilizing postcoat contains BSA; thus, no other antibody specificity is detectable in the assay. The anti- human IgM HRP conjugate specifically detects IgM and will not react with IgG, IgA, or IgE class antibodies.

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 **Wash Solution** and store at ambient temperature until kit is used entirely.
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°C until the kit lot expires or is used up.
Anti-Human IgM - HRP Conjugate Concentrate (100x) Part No. H-HuM.2a11, 0.15ml	Peroxidase conjugated anti- human IgM 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 2-8°C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
HIV-2 gp36 Microwell Strip Plate	HIV21	8-well strips (12)	Coated with recombinant HIV-2 gp36 and post-coated with stabilizers.
Anti-HIV-2 gp36 Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	HIV-22B HIV-22C HIV-22D HIV-22E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-HIV-2 gp36 in arbitrary activity Units; diluted in buffer with protein, detergents, and antimicrobial as stabilizers.
Anti-HIV-2 gp36 Positive Control	HIV-22PC	0.65ml	Anti-HIV-2 gp36 antibody; diluted in buffer with protein, detergents, and antimicrobial as stabilizers. [Value range on label]
Low NSB Sample Diluent	TBTm **Not** for conjugate dilution	30 ml	Buffer with protein, detergents, and antimicrobial as stabilizers. Use as is for sample dilution
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-Human IgM 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

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.

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 5 times the initial dilution and performed the same week as the assay; LNSD samples are stable frozen for several months.

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

ASSAY DESIGN

Review Interpretation of Results (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 1:200 or greater dilution for human serum with normal levels of IgG and IgM.
- Run the **Anti-HIV-2 gp36 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).
- 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 C.**
- 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 (18-30°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 (25-28°C). 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 **100 ul of blank, calibrators, samples, and controls** each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for **60 minutes at room temp** (25-28° C).
- **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 **100 ul of diluted Anti-Human IgM HRP** to each well.
- Incubate for **30 minutes at room temp**.
- **Wash wells 5** times as in step 1.

3. Substrate Incubation [100ul – 15 min]

- Add **100 ul 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 **100 ul 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.

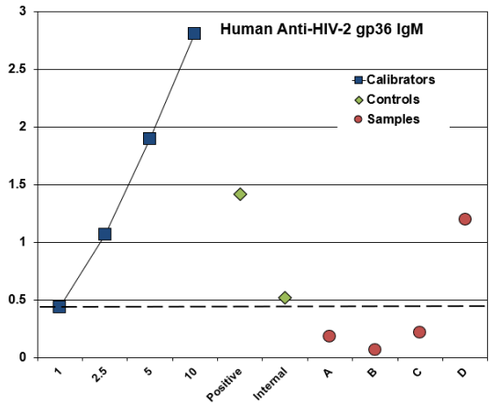
INTERPRETATION OF RESULTS

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-HIV-2 gp36 IgM, from either natural exposure 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 antiserum from anti-HIV-2 gp36 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 – an anti-HIV-2 gp36 serum; value range is on the vial label. This Control can be used to assess 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:

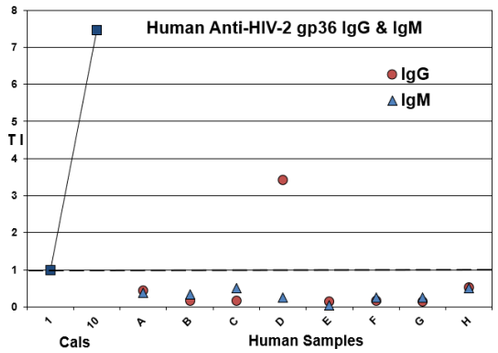
- Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

Example:

Human Serum IgG & IgM

A panel of human serum/plasma of unknown history was tested for anti-HIV-2 gp36 IgG & IgM (1:200 dilution in Low NSB Sample Diluent). **Threshold Index** was calculated using the **1 U/ml Cal**.



Results

Anti- HIV-2 gp36 IgG: all sera were negative (below the **1.0** Threshold Index) except for one (D) which was positive.

Anti- HIV-2 gp36 IgM: all sera were negative.

Notes:

1. **Positives** may be due to prior encounter with the virus or non-HIV proteins with common epitopes..

2. The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1:400) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1:100) 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).

3. When the **Positive Index** is above **5.0**, using a dilution curve to calculate titer is a more accurate quantitation method (see Method C).

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

   = **IgM Antibody Activity Units**

Calibrator Curve Quantitation

To quantitate antibody activity from a calibrator curve (such as provided with the kit), 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 cases of non-parallelism, antibody activity is best expressed as a titer relative to the titer of 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.

Instruction Manual No. M-HIV-120

Human Anti-HIV-2 gp36 IgM ELISA Kit

Cat. #HIV-120, 96 tests

For Quantitation of Anti-Human Immunodeficiency Virus (HIV) IgM in serum or plasma

For research use only, not for diagnostic or 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
www.4adi.com

ELISA Kit Components	Amount	Part
HIV-2 gp36 Coated Microwell Strip Plate	8-well strips (12)	HIV21
Anti-HIV-2 gp36 Positive Control	0.65 ml	HIV-22PC
Anti-HIV-2 gp36 Calibrator 1 U/ml	0.65 ml	HIV-22B
Anti-HIV-2 gp36 Calibrator 2.5 U/ml	0.65 ml	HIV-22C
Anti-HIV-2 gp36 Calibrator 5 U/ml	0.65 ml	HIV-22D
Anti-HIV-2 gp36 Calibrator 10 U/ml	0.65 ml	HIV-22E
Anti-Human IgM HRP (100X)	15 ml	H-HuM.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-HIV-120