Epitope Diagnostics, Inc.

HIV 1/2 ANTIBODY EIA KIT

Enzyme Linked Immunosorbent Assay for the Detection of Antibody Against HIV 1/2

Catalog Number: KT 1001 Store at 2 – 8ºC Upon Receipt

For Research Use Only. Not for Use in Clinical Diagnostic Procedures

INTENDED USE

This HIV ½ [Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2)] antibody EIA is a qualitative enzyme immunoassay for the detection of IgG subtype of antibody against HIV 1/2 in human serum or plasma. This kit is for research use only and must not be used for in vitro diagnostic purpose.

SUMMARY OF PHYSIOLOGY

Human immunodeficiency virus is a lentivirus (a member of the retrovirus family) that causes *acquired immunodeficiency syndrome* (AIDS). HIV infection in humans is considered pandemic by the World Health Organization (WHO). From its discovery in 1981 to 2006, AIDS killed more than 25 million people.

Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells. The four major routes of transmission are unsafe sex, contaminated needles, breast milk, and transmission from an infected mother to her baby at birth. Screening of blood products for HIV has largely eliminated transmission through blood transfusions or infected blood products in the developed world. HIV primarily infects vital cells in the human immune system such as helper T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells. HIV infection leads to low levels of $CD4^+$ T cells through three main mechanisms: firstly, direct viral killing of infected cells; secondly, increased rates of apoptosis in infected cells; and thirdly, killing of infected $CD4^+$ T cells by CD8 cytotoxic lymphocytes that recognize infected cells. When $CD4^+$ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections. Most people infected with HIV eventually develop AIDS. These individuals mostly die from opportunistic infections or malignancies associated with the progressive failure of the immune system.

HIV testing consists of initial screening with an enzyme-linked immunosorbent assay (ELISA) to detect antibodies to HIV. Specimens with a reactive ELISA result are retested in duplicate. If the result of either duplicate test is reactive, the specimen is reported as repeatedly reactive and undergoes confirmatory testing with a more specific supplemental test (e.g., Western blot or, less commonly, an immunofluorescence assay (IFA)). Nucleic acid testing (e.g., viral RNA or proviral DNA amplification method) can also help diagnosis in certain situations.

ASSAY PRINCIPLE

This HIV 1/2 Antibody ELISA is a serological test for the detection of HIV 1/2 antibody in human serum or plasma. Synthetic HIV 1/2 peptides and recombinant HIV 1/2 proteins corresponding to highly antigenic HIV 1/2 envelop glycoprotein and core proteins are coated onto the surface of each well of the microtiter plate. After contacting assay controls and test samples, human antibodies specific to HIV 1/2 are captured by binding to the specific HIV 1/2 antigens on the surface of the microwell, when human serum or plasma samples is added. Non-reactive antibodies and other proteins in the test sample are removed with wash solution. HIV 1/2 specific antibody are detected by incubating to a horseradish peroxidase conjugated (HRP) anti-human IgG antibody (Tracer Antibody). A solid phase captured immunocomplex is formed as "HIV antigen – Anti-HIV 1/2 Antibody – HRP conjugated Tracer Antibody". The test results are detected by subsequent reactions with a chromogenic substrate. Positive sample generates a medium to dark blue color. No color or very pale blue color indicates a negative reaction. The intensity of the reaction is photometrically measurable at OD 450 nm.

REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.

Materials provided with the kits:

Item	Description	96T	480T
1	HIV 1/2 Coated Microtiter Plate	1	5
2	HRP Conjugated Tracer Antibody (HIV 1/2)	12 ml	60ml
3	HIV 1/2 Antibody Negative Control	0.5 ml	2.5 ml
4	HIV 1/2 Antibody Positive Control	0.5 ml	2.5 ml
5	Wash Buffer Concentrate (20x)	40 ml	2X100 ml
6	Substrate Solution A	6 ml	30 ml
7	Substrate Solution B	6 ml	30 ml
8	Stop Solution	6 ml	30 ml
9	Patient Sample Diluent (HIV)	12 ml	60 ml

1. **HIV 1/2 Coated Microplate (Part # 30425)**
   Microtiter Plate (96 wells) coated with synthetic peptides and recombinant proteins corresponding to HIV 1/2 envelop glycoprotein and core proteins. This plate is of non-infectious. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. **HRP Conjugated Tracer Antibody (HIV) (Part # 30426)**
   This is the tracer antibody for the assay. One bottle contains HRP-conjugated anti-human IgG antibody in a stabilized matrix. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. **HIV 1/2 Antibody Negative Control (Part # 30427)**
   One vial contains HIV 1/2 Antibody Negative Control in a stabilized buffer. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. **HIV 1/2 Antibody Positive Control (Part # 30428)**
   One vial contains HIV 1/2 Antibody Positive Control in a stabilized buffer (Red colored). This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

5. **Wash Buffer Concentrate- 20 x (Part # 30275)**
   One bottle contains 20 fold concentrate. Before use the contents must be diluted 1:20with distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiration date on the kit box.

6. **Substrate Solution A (Part # 30277)**
   One bottle contains HRP Substrate. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

7. **Substrate Solution B (Part # 30278)**
   One bottle contains TMB Chromogen Substrate. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

8. **Stop Solution (Part # 30276)**
   One bottle contains 2N Sulfuric Acid. This reagent is ready to use. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

9. **Patient Sample Diluent (HIV) (Part# 30429)**
   One bottle contains ready to use phosphate buffered saline based buffer with bovine serum albumin added. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed pouch to minimize exposure to air. Use up the reagents as soon as possible after the kit is unpacked. Do not freeze any test reagents!

SAFTY PRECAUTIONS

The reagents must be used in research laboratory and are for research use only. Source material was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they are potential infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. Upon contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 20 µL, 25 µL, 50 µL, 100 µL, and 1000 µL.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm glass or plastic tubes.
5. Disposable plastic 1000 mL bottle with caps.
6. Aluminum foil.
7. Plastic microtiter well cover or polyethylene film.
8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
9. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

SPECIMEN COLLECTION

Either serum or plasma can be used in this test. No special preparation of individual is necessary prior to specimen collection. Whole blood should be collected by venipuncture and must be allowed to clot for a minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg for 10 minutes). The serum should be separated from the clot within two hours of blood collection and transferred to a clean test tube. Serum samples should be stored at 2 - 8ºC if the assay is to be performed within 24 hours. Otherwise, patient samples should be stored at – 20ºC or below until measurement. Avoid any repeated freezing and thawing of specimen.

ASSAY PROCEDURE

1. Reagent Preparation

(1) Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.
(2) ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.

3. Assay Procedure

(1) Place a sufficient number of HIV 1/2 antigen coated microwell strips in a holder to run assay controls and unknown samples in duplicate.
(2) Test Configuration

ROW	1	2	3	4	5	6	7
A	Blank	S2					
B	Neg. C	S2					
C	Neg. C	S3					
D	Neg. C	S3					
E	Pos. C	S4					
F	Pos. C	S4					
G	S1						
H	S1						

(3) Dispense **100 µl** of HIV 1/2 Antibody Negative and Positive Control into respective wells. Set one blank well as background control.
(4) Dispense **50 µl** of Patient Sample Diluent into each well that is designated for patient sample and Blank Well. **Do not dispense into the test control wells!**

(5) Dispense **50 µl** of neat unknown specimen into each designated sample well.
(6) Gently vortex or swirl the plate to mix well. Seal and incubate the plate **at 37°C for 40 min**.
(7) Wash each well 5 times by dispensing 350 µL of working wash solution (1x) into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(8) Add **100 µl** HRP Conjugate Tracer Antibody into each of the wells. Do not add Enzyme Conjugate to the blank well. Please add 100 µl washing buffer to the blank well.
(9) Gently vortex or swirl the plate to mix well. Seal and incubate the plate **at 37°C for 40 min**.
(10) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(11) Add **50 µl** of Substrate Solution A to each well, then immediately add **50 µl** of Substrate Solution B to each well. Mix gently and incubate **at 37°C for 20 min**.
(12) Add one drop (**50 µl**) of Stop Solution to each well to stop the color reaction. Read O.D. at 450 nm with an EIA reader.

PROCEDURAL NOTES

1. It is recommended that all controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light sensitive reagents in the original amber bottles.
3. Store any unused antibody coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading
7. All reagents should be mix gently and thoroughly prior use. Avoid foaming.

INTERPRETATION OF RESULTS

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):

Assay OD Quality Control: a valid assay must meet following conditions:

(1) The assay includes Blank, Negative Control and Positive Controls as outlined in the assay procedure.
(2) Each OD value of the Negative Controls must be less than or equal to 0.100 after subtracting the Blank.
(3) Each OD value of the Positive Controls must be greater than 0.600 after subtracting the Blank.

Cut-off Calculations:

Cut-off = Average OD values of Negative Controls + 0.10

Note: If the OD value of the negative control is less than 0.05, it should use 0.05 for calculation of the cut-off.

Unknown test sample results:

(1) Non-Reactive: Specimens with absorbance value less than the Cut-off value.
(2) Initial Reactive: Specimens with initial absorbance value greater than or equal to the Cut-off value. This specimen must be retested in duplicate before interpretation.
(3) Reactive: Initial Positive Specimen retested in duplicate shows a repeatedly Positive of either both wells or just one well.
(4) Non-Reactive: Initial Positive Specimen retested in duplicate shows negative in both wells.

IMPORTANT: Specimens which are repeatedly positive with this EIA should be further tested by additional and conformational tests!

LIMITATION OF THE PROCEDURE

1. Repeatedly reactive results in the HIV-1/HIV-2 EIA are presumptive evidence of antibodies in the specimen. AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically. Serological testing alone cannot be used to diagnose AIDS, even if the recommended investigation of reactive specimens suggests a high probability that the antibody to HIV-1/HIV-2 is present. RNA or DNA viral load test may provide further evidence and help in establishing clinical diagnosis.
2. The primary use of the HIV-1 and HIV-2 EIA is to screen blood and plasma donations so that units containing antibody can be identified and eliminated, or restricted to further manufacturing into non-injectable products.
3. A negative test result at any point in the investigation of individual subjects does not preclude the possibility of exposure to or infection with HIV-1/HIV-2.
4. As with all laboratory tests, a definitive clinical decision should not be made based only on the results of a single test. A complete evaluation by physician is needed.
5. Samples with initial reactive or initial positive or equivocal result must be re-analysed in duplicate. A reactive report must not be reported just based on initial reactive test result without repeated test in duplicate.
6. Optimal assay performance requires strict adherence to the assay procedure described. Deviation from the procedure may lead to aberrant results.
7. Do not use reagents from different tests that will cause incorrect results.
8. Following the procedure instruction closely, especially the incubation time and temperature.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known positive levels of HIV 1/2 antibody. We recommend that all assays include the laboratory's own control samples in addition to those provided with this kit.

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE

For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678. www.epitopediagnostics.com

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