Human Immunodeficiency Virus (HIV-1+2) (For in vitro diagnostic use only)

Store at 2 to 8°C

INTENDED USE
This HIV-1,2 ELISA Kit is to be used for the in vitro detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2) in serum or plasma. It is intended for initial detection of antibodies to HIV-1,2, not for determination.

INTRODUCTION
This HIV-1,2 ELISA Kit employs synthetic HIV polypeptides and recombinant HIV proteins for the detection of antibodies to HIV-1 and HIV-2. These polypeptides and recombinant proteins, which correspond to highly antigenic epitopes consisting of essential sequences derived from both the envelope and core proteins of HIV-1 and HIV-2, constitute the solid-phase antigenic adsorbent. HIV-1,2 ELISA Kit offers a sensitive, reliable and accurate interpretation of the reactivities to HIV with a 60 minute incubation period.

PRINCIPLE OF THE ASSAY
This HIV-1,2 Antibody enzyme linked immunosorbent assay (ELISA) kit employs a technique called a qualitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with synthetic HIV polypeptides and recombinant HIV proteins which correspond to highly antigenic epitopes consisting of essential sequences derived from both the envelope and core proteins of HIV-1 and HIV-2. Samples or controls are added to the microtiter plate wells and incubated. HIV specific antibodies, if present, will bind to and become immobilized by the antigen pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound HIV antibodies and other components of the sample. A standardized preparation of horseradish peroxidase (HRP)-conjugated synthetic HIV polypeptides and recombinant HIV proteins is added to each well to “sandwich” the HIV antibody immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugate and a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain HIV antibody and enzyme-conjugate will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm. Samples with O.D. values greater than or equal to the Cut-off Value are considered reactive by the criteria of this HIV-1,2 Antibody ELISA Kit.

Materials

Materials provided

1. Coated microplates:
   One microplate with 96 wells Pre-coated with HIV-1 & HIV-2 polypeptides and HIV recombinant proteins. Store at 2-8°C.

2. Enzyme Conjugate:
   One vial (7.5 ml) containing a solution of horseradish peroxidase (HRP) conjugated HIV-1 & HIV-2 polypeptides and HIV recombinant proteins. Store at 2-8°C.

3. Negative Control:
   One vial (1.0 ml) of inactivated normal human serum diluted in sample diluent. Store at 2-8°C.

4. Positive Control:
   One vial (1.0 ml) of inactivated HIV-1+2 antibody positive human serum diluted in sample diluent. Store at 2-8°C.

5. Substrate Solution A
   One vial (7.5 ml) containing Urea Hydrogen Peroxide. Store at 2-8°C.

6. Chromogen Solution B
   One vial (7.5 ml) containing stabilized 3,3'-5,5' Tetramethylbenzidine (TMB). Store at 2-8°C.

7. Stop Solution
   One vial (7.5 ml) containing 1.0 M H₂SO₄. Ready to use. Store at 2-8°C.

8. Washing solution (20X Conc.)
   One vial (50 ml) containing Phosphate Buffered Saline.

Preparation of the Working Washing Solution:
Into a graduated beaker of at least 1000ml capacity, pour the entire contents of the concentrated wash solution (50 ml). Rinse the vial with distilled water and pour also this distilled water into the becker up to a total volume of 1000ml. Transfer the contents of the becker into a 1500 ml bottle for storage.

Materials required but not provided

1. Precision Micropipettes and tips: 5-100ul
2. Incubator (+37°C)
3. Automatic plate washer
4. Microtiter plate reader for the measurement of absorbance at 450 nm
5. Adsorbent pad or paper
6. Distilled water
7. Vortex mixer
8. Disposable gloves.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Controls, conjugate, and microtiter plates are matched for optimal performance. Use only reagents supplied by manufacturer.

2. Allow kit reagents to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.

3. Do not use kit components beyond their expiration date.

4. Use only deionized or distilled water to dilute reagents.

5. Do not remove microtiter plate from storage bag until needed.Unused strips should be stored at 2-8 °C in its pouch with the desiccant provided.

6. Use fresh disposable pipette tips for each transfer to avoid contamination.

7. Do not mix acid and sodium hypochlorite solutions. Human serum and plasma should be handled as
8. Potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All materials should be disposed of in a manner that will inactivate human viruses.
10. Solid Waste: autoclave 60 min. at 121°C. Liquid Waste: add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand a minimum of 30 minutes to inactivate the viruses before disposal.
11. Substrate B contains 20% acetone, keep reagent away from flaming source.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION, COLLECTION, HANDLING, AND STORAGE

Serum: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.
Plasma: Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection the specimen must be kept at 4°C to remove any particulates.

1. This HIV-1,2 Antibody ELISA Kit is not affected by haemolysis of specimens. No adverse effects have been noted in the presence of anti-coagulants, sodium citrate, EDTA, or heparin.
2. Avoid grossly hemolytic, lipidic or turbid samples.
3. Serum or plasma samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
4. When performing the assay slowly bring samples to room temperature.
5. It is recommended that all samples be assayed in duplicate.
6. Do not use the treated specimens.

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 bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

TEST PROCEDURE
1. Bring all reagents and specimens to room temperature (15°C-30°C) before beginning the assay. Swirl gently before use. Adjust the incubator at +37°C, if necessary.
2. Write down the relative numbers of specimens and wells on the data sheet. One well for the blank, five additional wells for the controls and one well for each specimen. If the experiment does not need the whole plate, remove the remaining strips from the strip holder and store the unused strips in the ziplock bag (included in the kit) which contains desiccant.
3. Reserve one well for blank, add 50ul of the Negative Control to each of three wells, 50ul of the Positive Control to each of two wells, and 50ul of each specimen to one well according to the following scheme:

   1 A : Blank
   2 A, 3 A, 4 A : Negative Control Serum
   5 A, 6 A : Positive Control Serum
   7 A : Samples

   Notes: use an individual tip for each pipetting to avoid cross contaminations.
   If more than 2 or 3 runs per plate are required and reliable apparatus (i.e. pipettes, washers, etc.) are used, one can pipette the Negative Control in duplicate and the Positive Control in singleton.
4. Incubate the plate in a +37°C incubator for 30 minutes.
5. At the end of the incubation wash the strips 6 times with the working wash solution either manually or with an automatic washer. After the final washing, make sure that whole solution is perfectly removed from each well.

Note: Proper washing procedure is essential for good assay performance.

6. Add 50 ul of Enzyme Conjugate to each well (except blank well). Mix it gently by swirling the microtiter plate on flat bench. Incubate for 30 minutes at +37°C.
7. Wash the plate 6 times as step 5.
8. Dispense 50ul of Chromogen Substrate A and B, including 1A. Mix horizontally and incubate for 10 minutes at +37°C (avoid exposure to direct sunlight).
9. Stop the reaction by adding 50 ul of blocking reagent to each well (including the blank) in the same order adopted for the addition of reagents. Incubate the plate for 30 minutes.
10. After adding the stop solution, read the color developed on the microplate reader at 450 nm. The reading should be done within 30 min. from the stop.

Note: The reader should be blanked at 450 nm against the blank. Bichromatic absorbance measurement with a reference wavelength of 620nm is recommended when available.

11. Record the absorbance results on a data sheet. Include the kit lot number, date, operator and any notes about the run. If a printed copy of the absorbance reading is available, it should be attached to the data sheet.

WASHING PROCEDURE

Microtiter plates could be washed manually or automatically, in which case it is absolutely necessary to program the microplate washer according to the following directions: Aspirate the incubation mixture from the wells of the first strip and dispense 350 ul of washing solution. Soon afterwards...
process the other strips in the same way. Repeat this procedure for 5 further times, for a total of 6 cycles, making sure that the washing solution remains in the wells for 30 sec. (soaking time) each cycle. Proper wash procedure is essential for a good assay performance.

**TEST VALIDITY**

Test is valid if:

a) Mean negative control is lower than 0.10 O.D.
b) Mean positive control is equal to or higher than 0.70 O.D.
c) (Mean positive control O.D) - (Mean negative control O.D) > 0.60

**DETERMINATION OF CUT-OFF VALUE AND INTERPRETATION OF SAMPLES RESULTS**

Cut-off value = \(0.1 + N \times (\text{Mean Negative Control})\)  

If the O.D. value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual O.D. value measured.  

Example: NCx = 0.032  
Cut-off = 0.1 + 0.005 \(= 0.105\)

Any sample, in which the absorbance is equal to or higher than the Cut-off value, is considered Positive for HIV. Any sample, in which the absorbance is lower than the calculated Cut-off value, is considered Negative for HIV.

**INTERPRETATION OF RESULTS**

1. Samples with O.D. values LESS THAN the CUT-OFF value are considered NON-REACTIVE for antibodies to HIV-1,2 by the criteria of this HIV-1,2 Antibody ELISA Kit.
2. Samples with O.D. values GREATER THAN or EQUAL to the CUT-OFF value are considered INITIALLY REACTIVE for antibodies to HIV-1,2 by the criteria of the HIV-1,2 Antibody ELISA Kit. The original sample should be rediluted and retested, in duplicate before interpretation.
3. Initially reactive samples which are found to be non-reactive on retesting are considered NEGATIVE by the criteria of this HIV-1,2 Antibody ELISA Kit.
4. Samples that are found reactive on retesting are interpreted to be REPEATEDLY REACTIVE for antibodies to HIV-1,2 by the criteria of this HIV-1,2 Antibody ELISA Kit.
5. If a sample is found repeatedly reactive, there is a high probability that it contains antibodies to HIV-1 and/or HIV-2, especially if it is from a patient that is at high risk for infection or the O.D. value is very high. It is recommended that repeatedly reactive samples be investigated by more specific tests such as Western Blot, immunofluorescence, or radioimmunoprecipitation which are capable of identifying antibodies specific to HIV-1,2.

**SENSITIVITY & SPECIFICITY:**

HIV-1 and HIV-2 sensitivity is 100% for detecting antibodies in individuals infected with HIV-1 and/or HIV-2. Specificity based on assumed zero prevalence rate of antibodies to HIV-1 and HIV-2 in random donors is 99.7%.

**LIMITATIONS OF THE PROCEDURE**

The Assay Procedure and the Interpretation of Results sections must be closely adhered to when testing the serum or plasma of individual subjects for the presence of antibodies to HIV. This HIV-1,2 ELISA was designed to test individual units of serum or plasma, thus most data regarding its interpretation was derived from testing individual samples. There is insufficient data to interpret tests performed on other body fluids, such as pooled blood or processed plasma, and products made from such pooled specimens. Testing of such samples is not recommended.

This HIV-1,2 ELISA detects antibodies to HIV-1 and/or HIV-2 in serum and plasma. Clinical studies continue to clarify and refine the interpretation and medical significance of the presence of antibodies to HIV. For most uses it is recommended that repeatedly reactive samples be investigated by supplemental tests. It has been suggested that repeatedly reactive specimens obtained from populations at increased risk and at low risk for HIV infection are more likely to demonstrate the presence of HIV antibodies with supplemental testing, such as Western Blot, immunofluorescence or radioimmuno-precipitation. Reactivity at or only slightly above the cutoff-value is more frequently non-specific, especially from persons at low risk for HIV infection. A person who has antibodies to HIV is presumed to be infected with the virus and appropriate counselling and medical evaluation should be offered. Such evaluation should be considered an important part of HIV antibody testing and should include test result confirmation on a freshly drawn sample.

AIDS and AIDS-related conditions are clinical syndromes and their diagnosis can only be established clinically by a physician. ELISA testing alone cannot be used to diagnose AIDS, even if the recommended investigation of reactive samples suggests a high probability that the antibody to HIV is present. 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. The risk of an asymptomatic person with a repeatedly reactive serum developing AIDS is not known.

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