Human anti-HIV 1+2 ELISA Kit

Cat.No: IVDEIA002
Lot. No. (See product label)

Size

96T

Intended use

The Human anti-HIV 1+2 ELISA Kit is an enzyme-linked immunosorbent assay (ELISA) intended for qualitative detection of antibodies to Human Immunodeficiency Viruses (HIV) type 1 or type 2 in human serum or plasma samples. The assay can be utilized for screening of blood donors and/or as an aid in the diagnosis of clinical conditions related to infection with HIV-1 and/or HIV-2 - the etiological agents of the acquired immunodeficiency syndrome (AIDS).

General Description

Serological evidence of infection with HIV may be obtained by testing for presence of HIV antigens or antibodies in serum of individuals suspected for HIV infection. Antigen can generally be detected during both acute phase and the symptomatic phase of AIDS only. The antibodies to HIV-1 and/or HIV-2 can be detected throughout virtually the whole infection period, starting at or shortly after the acute phase and lasting till the end stage of AIDS. Therefore, the use of highly sensitive antibody assays is the primary approach in serodiagnosis of HIV infection. Apart from sexual transmission, the principal route of infection with HIV is blood transfusion. HIV can present both in cellular and cell-free fractions of human blood. Therefore, all donations of blood or plasma should be tested because due to the risk of HIV transmission through contaminated blood. This can be effectively achieved by testing for the antibodies to HIV-1 and HIV-2 by using a highly sensitive ELISA tests.

Principle Of The Test

Human anti-HIV 1+2 ELISA Kit is a two step incubation antigen "sandwich" enzyme immunoassay kit, which uses polystyrene microwell strips pre-coated with recombinant HIV antigens expressed in E.coli (recombinant HIV-1 and recombinant HIV-2). Patient's serum or plasma sample is added, and during the first incubation step, the specific HIV1/2 antibodies will be captured inside the wells if present. The microwells are then washed to remove unbound serum proteins. A second set of recombinant antigens conjugated to the enzyme Horseradish Peroxidase (HRP-Conjugate) and expressing the same epitopes as the pre-coated antigens is added, and during the second incubation, they will bind to the captured antibody. The microwells are washed to remove unbound conjugate, and Chromogen solutions are added into the wells. In wells containing the antigen-antibody-antigen (HRP) "sandwich" immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after the reaction is stopped with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HIV 1/2 remain colorless.

Reagents And Materials Provided

1. MICROWELL PLATE: 1x96wells
2. HRP-CONJUGATE: 1x12ml per vial,
3. POSITIVE CONTROL-1: 1x1ml per vial,
4. POSITIVE CONTROL-2: 0.5x1ml per vial
5. NEGATIVE CONTROL: 1x1ml per vial,
6. WASH BUFFER(25x): 1x80ml per bottle
7. CHROMOGEN SOLUTION A: 1x8ml per vial
8. CHROMOGEN SOLUTION B: 1x8ml per vial
9. STOP SOLUTION: 1x7ml per vial
10. CARDBOARD PLATE COVER (5 sheets)
11. PACKAGE INSERT: 1

Materials Required But Not Supplied

1. Distilled water.
2. Manual or automatic pipettors capable of delivering 25μL- 200μL.
3. Disposable pipette tips.
4. Timer.
5. Microplate mixer.
6. Incubator 37°C.
7. Microplate washer (alternatively, washing can be performed manually, e.g. by using a repeating syringe delivering 0.3ml volumes).
8. Microplate reader equipped with a 450nm and 630nm filter.

Storage

1. If kept at 2 to 8°C, all the test reagents are stable until the expiry date printed on the kit.
2. When the aluminum bag has been opened, the unused strips can be safely stored at 2-8°C in the sealable plastic pouch along with the silica gel placed inside for about two weeks.

Specimen Collection And Preparation

Human anti-HIV 1+2 ELISA is intended ONLY for testing of individual serum or plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
Storage: Store specimens at 2-8°C. Specimens not required for assaying within 3 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided.

Reagent Preparation

Allow the reagents to reach room temperature (18-30°C) for 15-30 minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute (1:25) the Wash buffer as indicated in the washing step instructions. Use distilled or deionized water and only clean vessels to dilute the buffer. All other reagents are READY TO USE AS SUPPLIED.

Assay Procedure

1. Preparation: Mark three wells as Positive control (two for HIV-1 and one for HIV-2), one well as Negative control and one
Blank (neither samples or HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

2. Adding Sample: Add 100μl of samples, and 100μl Positive and Negative controls into their respective wells. Note: Use a separate disposable pipette tip for each specimen, Negative and Positive Control as to avoid cross-contamination.

3. Incubating Sample: Cover the plate with the plate cover and incubate for 60min at 37°C. It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during incubation. If dry incubator is used, do not open the door frequently.

4. Washing: After the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Washing buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.

5. Adding HRP-Conjugate: Add 100μl of HRP-Conjugate reagent into each well except for the Blank.

6. Incubating HRP-Conjugate: Cover the plate with the plate cover and incubate for 30min. at 37°C.


8. Coloring: Add 50μl of Chromogen A and 50μl Chromogen B solutions into each well including the Blank. Incubate the plate at 37°C for 30 min avoiding light.

9. Stopping Reaction: Using a multichannel pipette or manually, add 50μl Stop solution into each well and mix gently.

10. Measuring the Absorbance: Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 10minutes after stopping the reaction).

### Interpretation Of Results

**Negative Results (S/COV < 1):** Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no anti-HIV 1/2 antibodies have been detected with CD anti-HIV 1+2 ELISA kit, therefore the patient is probably not infected with HIV 1/2 and the blood unit do not contain antibodies to HIV 1/2 and could be transfused in case that other infectious diseases markers are also absent.

**Positive Results (S/COV ≥ 1):** Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that anti-HIV 1/2 antibodies have probably been detected using CD anti-HIV 1+2 ELISA. All initially reactive specimens should be retested in duplicates using CD anti-HIV 1+2 ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for antibodies to HIV 1/2 with CD anti-HIV 1+2 ELISA

### Evaluation

**Abbreviations**

- COV: Cut Off Value
- NC = the mean absorbance of the negative controls
- PC1 = the mean absorbance of the positive control 1
- PC2 = the mean absorbance of the positive control 2
- PCx = (PC1+PC2) / 2
- S = the absorbance of the test sample

The test results are valid if the Quality Control criteria are fulfilled:

1. NC<0.08
2. PCx>0.80, PC2>0.30
3. Dual wavelength - the mean absorbance of blank < 0.04; Single wavelength - the mean absorbance of blank < 0.08.

COV = PCx X 10%

Note: when (PC1+PC2)/2>2.5, PCx=2.5
Precautions

TO BE USED ONLY FROM QUALIFIED PROFESSIONALS

The ELISA assays are time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.

2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.

3. CAUTION - CRITICAL STEP: Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2-8°C immediately after use.

4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.

5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.

6. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air bubbles when adding the reagents.

7. Avoid assay steps long time interruptions. Assure same working conditions for all wells.

8. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.

9. Assure that the incubation temperature is 37°C inside the incubator.

10. When adding specimens, do not touch the well's bottom with the pipette tip.

11. When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.

12. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids and alkalis. Do not perform the assay in the presence of these substances.

13. If using fully automated equipment, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

14. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.

15. WARNING: Materials from human origin may have been used in the preparation of the Negative Control of the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.


17. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.

18. The pipette tips, vials, strips and specimen containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should NEVER be autoclaved. Materials Safety Data Sheet (MSDS) available upon request.

19. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.