HIV 1+2 Antibody ELISA Kit

- **96-well ELISA kit for the Qualitative Detection of IgG + IgM + IgA to HIV 1+2 virus in Human Serum, plasma**
- **For Export Only, Not for Re-sale in the USA.**
- **Not for Internal or External Use in Humans or Animals.**
- **Store at 2 - 8ºC Upon Receiving.**

INTENDED USE

The HIV 1+2 Antibody ELISA kit is an enzyme linked immunosorbent assay (ELISA) for the qualitative determination of HIV 1+2 antibodies (IgG+IgM+IgA) in human serum or plasma. It is intended for professional use only. ***Positive result must be confirmed by confirmative tests.***

INTRODUCTION

Human immunodeficiency virus type I and type II (HIV1+2) are enveloped single strain RNA positive virus. The causative relationship between HIV1+2 virus and acquired immunodeficiency syndrome (AIDS) has been established over decades. HIV-1 has been isolated from patients with AIDS and AIDS-related complex, and from healthy individuals with a high risk for developing AIDS (1). HIV-2 has been isolated from West African AIDS patients and from seropositive asymptomatic individuals (2).

Both HIV-1 and -2 virus can elicit strong immune responses (3), including the production of anti virus antibodies. Presence of specific anti HIV 1 and or II virus antibody in serum or plasma indicates the exposure of an individual to the HIV-1 and or -2 virus, being a great value of clinical diagnosis (4).

The HIV 1+2 ELISA kit is a double antigen-sandwich, two-step wash assay. It is highly sensitive and specific. In addition, it eliminates the hook effect in the high titer sample which exists in the one-step wash assay.

TEST PRINCIPLE

The HIV 1+2 ELISA kit (Serum/Plasma) utilizes two pairs of HIV 1 and 2 recombinant antigens to detect HIV antibodies qualitatively and selectively in serum or plasma. The test microwell was coated with HIV 1and 2 recombinant envelope antigens. The HIV-1and or 2 antibodies, if present in the test sample, bind to the recombinant antigens immobilized on the microwell surface. After the first wash, the bound antibodies was then incubated with horseradish peroxidase enzyme (HRP) conjugated HIV type 1 and 2 recombinant envelope antigen (HIV-HRP conjugate), forming an antigen-antibody- antigen enzyme complex on the microwell surface. Upon addition of TMB substrate, the bound enzyme generates color. The intensity of the color is directly proportional to the concentration of HIV 1 and or 2 antibodies in the samples.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

1. 12 x8-well strips coated with recombinant HIV 1+2 antigens (*Cat#E0410W)*
2. 6 ml HRP-HIV antigen conjugates (*Cat#E0410H)*
3. 1 ml HIV antibody negative control (*Cat#E0410N)*
4. 1 ml HIV antibody Positive Control (*Cat#E0410P)*
5. 20 ml Wash Buffer (30 x concentrate) (*Cat#WE3000)*
6. 11 ml TMB substrate (*Cat#TME2000*)
7. 6 ml Stop solution (*Cat#SE1000)*
8. 1 set Product insert
9. 2 sets ELISA Working Sheet

Materials and reagents required but not provided in the kit

ı. Pipette capable of delivering 1-50 ul volumes with a precision better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 50ul and 200ul ml volumes with a precision better than 1.5%.
3. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nmwavelength is acceptable
4. Absorbent paper for blotting the microplate wells.
5. Parafilm or other adhesive film sealant for sealing plate.
6. Timer.

STORAGE AND STABILITY

- Test components are stable up to their expiration data when stored at 2º-8ºC. Do not freeze.
- Return all reagents requiring refrigeration immediately after use. Reseal the microwells after removing the desired number of wells.
- Do not mix or use components from the kits with different lot numbers. Do not use reagents after their expiration date.
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WARNING AND PRECAUTIONS

The test kit does not contain any viable infectious agents. However all patient samples must be considered as potentially infectious.

- Do not ingest the reagents. Avoid contact with eyes, skin and mucose. Wear protective clothing and disposable gloves. Do not allow smoking or eating where antigen containing materials are being handled.
- Preclude any pipetting by mouth.
- Handle all patient samples and test components as though capable of transmitting infection. Clean spills thoroughly using an appropriate intermediate-to high level disinfectant. Decontaminate and dispose of specimens and all potentially contaminated materials as if they contain infectious agents.
- Avoid splashing or aerosol formation.
- Avoid contacting Substrate Reagent or Stop solution with the skin or other mucose membranes. In case of contact, wash thoroughly with water.

SPECIMEN COLLECTION AND PREPARATION

- Serum should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
- This kit is designed for use with serum sample without additives only.
- If a specimen is not tested immediately, refrigerated at 2º-8ºC. If storage period greater than three days are anticipated, the specimen should be frozen (-20ºC). Avoid repeated freezing-thawing of samples. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.
- Do not use serum samples demonstrating gross lipermia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PROCEDURE NOTES

- Reliable and reproducible results will be obtained when The assay procedure is carried out with a complete understanding of the package insert instruction.
- Do not splash liquid while rocking or shaking the wells.
- The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.
- Avoid strong light during color development. In the beginning of each incubation and after adding Stopping Solution, gently rocking the microwells to ensure thorough mixing. Avoid the formation of air bubbles as which results in inaccurate absorbance values.

ASSAY PROCEDURE

A: Preparation

1. Bring all reagents, controls to room temperature (18-25˚C).
2. Dilute concentrated Washing Buffer 30 fold with distilled water. Add 580 ml of distill water to 20 ml concentrated Washing Buffer and mix well. Warm up the concentrated washing buffer if precipitants appear.
3. Mix each reagent before adding to the test wells.
4. Determine the number of microwells needed and mark on the ELISA Working Sheet with the appropriate information. Positive, and Negative Controls require be run in duplicate to ensure accuracy. Blank well is not necessary, an optional.

B: Assaying

1. Remove the desired number of strips and re-seal un-used strips.
2. Add **50 ul** of positive, negative control into appropriate wells.
3. Add **50 ul** of test samples into the test well.
4. Incubate the wells at **37°C for 30 minutes**.
5. Carefully remove the incubation mixture by empting the solution into a waste container. Fill each well with diluted wash buffer and shake gently for 20-30 second. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 4 more times.
6. Add **50 ul** (one drop) of HIV-HRP conjugates into each well expect the blank well. Gently rock the wells for twenty second, then cover the wells.
   Note: For better precision, please use pipette to transfer the solution.
7. Incubate the wells at **37°C for 30 minutes**.
8. Carefully remove the incubation mixture by empting the solution into a waste container. Fill each well with diluted wash buffer and shake gently for 20-30 second. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 4 more times.
9. Add **100 ul** (or two drops) of TMB substrate solution each well, then incubate for **15 minutes at room temperature** in dark if possible.
10. Stop the reaction by adding **50 ul** (one drop) of stop buffer to each well. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
11. Set the microplate reader wavelength at **450 nm** and measure the absorbance of each well within 15 minutes after adding Stop Solution. A filter of 620 -690nm can be used as a reference wavelength to optimize the assay result.

INTERPRETATION OF RESULTS

A: Set up the cutoff value

The cutoff value = 0.10 + N

N: Mean OD of the negative control. Use 0.05 for calculation of the cutoff value if less than 0.05.

B. Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the Cutoff Value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cutoff Value}}$$

C. Interpretations

Specimen OD ratio	
Negative	< 1.00
Positive	≥ 1.00

- The negative result indicates that there is no detectable anti HIV1/2 antibodies in the specimen by the HIV 1+2 ^{BLUE}ELISA kit. However, it is strongly recommended to dilute the specimen at 1:100 dilution with Sample Diluent and repeat assay if the specimen is highly suspicious and its initial result is negative.
- The specimen with a positive result should be tested duplicate again and confirmed with Western blot or other tests.

D. Quality Control

The mean OD value of the positive controls should be ≥ 0.50.
The mean OD value of the negative controls should be ≤ 0.10.

Check the procedure and repeat assay if above conditions are not met.

PERFORMANCE CHARACTERISTIC

Sensitivity: The HIV 1+2 ELISA kit has detected all the positive samples in the BBI Anti-HIV 1 Low Titer Performance Panel (PRB 108).

Specificity: No false positive was observed by the HIV 1+2 ELISA kit from 450 normal sera.

Hook Effect: No hook effect was observed with samples at antibody titer as high as 1:10,000. It is unknown if hook effect appears with samples at titer higher than 10,000 due to shortage of such samples.

LIMITATION OF THE TEST

1. The HIV 1+2 ^{BLUE}ELISA kit is limited to the detection of anti HIV 1/2 antibodies in human serum or plasma.
2. The test is a qualitative screening assay and is not for determining quantitative concentration of HIV 1/2 virus antibodies.
3. A negative result does not rule out HIV 1/2 infection because the antibodies to HIV 1/2 may be absent at the time the specimen is taken or may not be present in sufficient quality to be detected at early stage of infection.
4. **If the initial result of a highly suspicious sample is negative, dilute the sample at 1:100 dilution with the Negative Control provided in the kit and retest.**
5. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after evaluation of all clinical and laboratory findings.

REFERENCES

1. Chang, SY, Bowman, BH, Weiss, JB, Garcia, RE and White, TJ. *The origin of HIV-1 isolate HTLV-IIIB*. Nature (1993)3/363:466-9
2. Arya, SK, Beaver, B, Jagodzinski, L, Ensoli, B, Kanki, PJ, Albert, J, Fenyo, EM, Biberfeld, G, Zagury, JF and Laure, F. *New human and simian HIV-related retroviruses possess functional transactivator (tat) gene.* Nature (1987) 328:548-550
3. Caetano JA *Immunologic aspects of HIV infection*. Acta Med Port (1991) 4 Suppl 1:52S-58S
4. Janssen, RS, Satten, GA, Stramer, SL, Rawal, BD, O'Brien, TR, Weiblen, BJ, Hecht, FM, Jack, N, Cleghorn, FR, Kahn, JO, Chesney, MA and Busch MP. *New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes.* JAMA (1998) 280(1): 42-48