Human HIV(1+2 antibodies) (3rd Generation) ELISA Kit

Catalogue No.: EH4100

Size: 96T

Reactivity: Human

Application: This immunoassay kit allows for the qualitative determination of HIV1/HIV2 Ab in human serum or plasma.

Storage: 2-8℃

Expiry Date: see kit label

Principle: Sandwich

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(96T)	Storage
Micro ELISA Plate(Dismountable)	8 ×12	2-8℃
HIV-1-Ab Positive Control	1 vial	2-8℃
HIV-2-Ab Positive Control	1 vial	2-8℃
HIV-Ab Negative Control	1 vial	2-8℃
HRP- Conjugates antigen	1 vial	2-8℃(Avoid Direct Light)
TMB substrate A	1 vial	2-8℃(Avoid Direct Light)
TMB substrate B	1 vial	2-8℃(Avoid Direct Light)
Stop solution	1 vial	2-8℃
Wash buffer (20X)	25ml×2	2-8℃
Plate Sealer	5pieces	
Product Description	1 copy	

Background

Present evidence indicates that Acquired Immunodeficiency Syndrome is caused by HIV-1 and HIV-2. The viruses are transmitted by sexual contact, exposure to blood (including sharing contaminated needles and syringes) or certain blood product, or transmitted from an infected mother to her fetus or child during the prenatal period. Presence of antibodies to the virus in the serum of a patient indicates viral infection. Human HIV (1+ 2 antibodies) ELISA Kit is a third generation Enzyme Linked Immunosorbent Assay (ELISA) which can detect anti HIV antibodies.

Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Recombinant HIV(1+2) antigens pre-coated on onto 96-well plates. The controls, test samples and HRP- Conjugates were added to the wells subsequently, and wash with wash buffer. If any specific HIV antibodies are present in the sample, they will be captured inside the wells at the time of first incubation. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.

Precautions for Use

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.

2. After opening and before using, keep plate dry.

3. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.

4. Storage TMB reagents avoid light.

5. Washing process is very important, not fully wash easily cause a false positive.

6. Duplicate well assay is recommended for both standard and sample testing.

7. Don't let Micro plate dry at the assay, for dry plate will inactivate active components on plate.

8. Don't reuse tips and tubes to avoid cross contamination.

9.Please do not mix the reagents in different kits of our company. Do not mix reagents from other manufacturers.

10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Material Required But Not Supplied

1. Microplate reader (wavelength: 450nm)

2.37℃ incubator

3. Automated plate washer

4. Precision single and multi-channel pipette and disposable tips

5. Clean tubes and Eppendorf tubes

6. Deionized or distilled water

Manual Washing

Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of TWO washes.

Automated Washing

Aspirate all wells, then wash plate TWO times with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20℃ for long term. Avoid multiple freeze-thaw cycles.

- Serum: Coagulate the serum at room temperature (about 1 hour). Centrifuge at approximately 1000 ×g for 15 min. Analyze the serum immediately or aliquot and store at -20℃.

- Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15min at 2-8 ℃ at 1500 x g within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8 ℃ at 10000 x g. Analyze immediately or aliquot and store frozen at -20℃.

Notes: Samples to be used within 5 days can be stored at 2-8℃. Otherwise, samples should be stored at -20℃, -80℃ or under liquid nitrogen condition to avoid loss of bioactivity and contamination. Avoid repeated freeze-thaw cycles. Severely hemolyzed samples are unsuitable for this assay.

Sample requirements

1. Serum or plasma samples should be taken, and the plasma samples can be treated with heparin sodium citrate or EDTA anticoagulant
2. The samples should not be preserved with sodium azide to avoid inhibiting enzyme activity.
3. If the samples are stored at 2~8 ℃ for a long time, they should be frozen at -15 ~ 20 ℃ to avoid repeated freeze-thaw
4. Hyperlipidemia, hyperbilirubin and hemolysis samples may affect the accuracy of test results, so it is recommended not to use

Reagent Preparation and Storage

Wash Buffer:

Dilute 50 mL Concentrated Wash Buffer into 1000 mL Wash Buffer with deionized or distilled water. Put unused solution back at 4 ℃. If crystals have formed in the concentrate, you can warm it with 40 ℃ water bath (Heating temperature should not exceed 50 ℃) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

Assay Procedure

Put the kit at room temperature for 20 minutes before use

1. Label the sample wells, Negative Control, Positive Control and blank well.
2. Add 50 μL of each control or Samples to appropriate wells of the microtiter plate
3. Seal the plate with a cover and incubate at 37 ℃ for 60 min.
4. Remove the cover and wash plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 0.5-1 min.

5. Add 100ul of HRP- Conjugates antigen into the above wells (controls, test sample). Add the solution at the bottom of each well without touching the side wall.

6. Seal the plate with a cover and incubate at 37℃ for 30 min.

8. Remove the cover and wash plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 0.5-1 min.

9. Add 50 μl of TMB substrate A and 50 μl of TMB substrate B into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37℃ in dark within for 30 min.

10. Add 50 μl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

11. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution. (Use the blank well to set zero)

Data Analysis

Calculation of the Cutoff Value

Cutoff Value = NCx +0.1

NCx (Mean Absorbance of Negative Control). PCx (Mean Absorbance of Positive Control)

when NCx<0.05, Calculate as 0.05.

Calculation of Results

1. Sample with absorbance values < Cutoff Value are NON-REACTIVE and are considered NEGATIVE.

Sample with absorbance value ≥ Cutoff Value are considered INITIALLY REACTIVE.

2. If NCx>0.1 or PCx≤0.6, the test is regarded as Invalid, should be tested again.

Notes: Those positive in the initial screening should take a new sample for double-hole retest, and those positive in the retest should be sent to the HIV confirmation laboratory for related confirmation tests in accordance with the national HIV testing management norms.