Human HIV(1+2 antibodies plus p24 antigen)(4th Generation) ELISA Kit

Catalogue No.: EH4101

Size: 96T

Reactivity: Human

Application: For qualitative determination of HIV-1 p24 antigen and HIV-1/HIV-2 antibody in human serum or plasma.

Storage: 2-8℃

Expiry Date: see kit label

NOTE: FOR RESEARCH USE ONLY.

Kit Components:

Item	Specifications(96T)	Storage
Micro ELISA Plate(Dismountable)	8 ×12	2-8℃
HIV-1-Ab Positive Control	1ml	2-8℃
HIV-2-Ab Positive Control	1ml	2-8℃
HIV-Ag Positive Control	1ml	2-8℃
HIV-Ag/ Ab Negative Control	1ml	2-8℃
HRP- Conjugates antigen/HRP-SA	12ml	2-8℃(Avoid Direct Light)
Biotin- Conjugates antibody	5ml	2-8℃(Avoid Direct Light)
TMB substrate A	14ml	2-8℃(Avoid Direct Light)
TMB substrate B	14ml	2-8℃(Avoid Direct Light)
Stop solution	14ml	2-8℃
Wash buffer (20X)	25ml×2	2-8℃
Plate Sealer	3pieces	
Product Description	1 copy	

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Operation Procedure

Principle of the Assay

This kit was based on enzyme-linked immune-sorbent assay technology to detect HIV-1 p24 antigen(Double-antibody sandwich ELISA, DAS-ELISA) and HIV antibody(Double-antigen sandwich ELISA, DAgS-ELISA) in human serum or plasma. Anti-HIV (p24) monoclonal antibody and recombinant HIV antigen are pre-coated on 96-well plate. Biotin-labeled P24 antibody, HRP-streptavidin conjugate, and recombinant HRP-conjugated HIV antigen are also included. If the sample contains HIV-1 p24 antigen, the immunocomplex "immobilized HIV-1 p24 antibody - HIV-1 p24 antigen - biotin-labeled p24 antibody - streptavidin-horseradish peroxidase (SA-HRP)" forms during the reaction. The TMB substrate is added to visualize HRP enzymatic reaction. If the sample contains HIV antibody, the immunocomplex "immobilized HIV antigen - HIV antibody - enzyme-labeled antigen" forms during the reaction. The staining reaction also occurs after adding the TMB substrate.

Precautions

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 and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate 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°C 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

Washing Method

Manual: 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.

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Automatic: Aspirate all wells, and then wash plate 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 shall be set for soaking 1 minute. (**Note**: set the height of the needles; be sure the fluid can be sipped up completely)

Sample Collection and Storage(Universal)

- **Serum:** Place all serum samples at room temperature for 2 hours or at 2 - 8℃ overnight and centrifuge for 20min at approximately 1000xg. Collect the supernatant to perform the assay immediately. Disposable blood collection tubes without pyrogen and endotoxin are required.
- **Plasma:** Collect plasma with EDTA-Na2 or heparin as anticoagulant. Centrifuge for 15min at 2-8℃ at 1000 x g within 30 min of collection. Collect the supernatant to perform the assay immediately. Hemolyzed samples with high cholesterol should be avoided.

Sample Requirements: Collected serum or plasma sample should be stored under sterile condition. Anticoagulant samples applicable to heparin, sodium citrate or EDTA can be used in this assay.

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.

Reagent Preparation and Storage

Place all reagents and samples at room temperature for 30min before use.

Wash Buffer:

Dilute 50mL concentrated wash buffer into 1000mL wash buffer with deionized or distilled water and mix thoroughly. (The recommended resistivity of ultrapure water is 18MΩ.)

Assay Procedure

1. Take required amount of strips. Then fix strips on the plate and number them. Set 3 negative control well. Set 1 positive control well for antigen, HIV-1 antibody and HIV-2 antibody respectively. Set 1 blank control well and leave it empty(Don't add samples, biotin reagents and HRP-Conjugates).
2. **Add biotin reagents:** Add 25ul biotin reagents in the appropriate well.
3. **Sample loading**: Add 75ul negative and positive control serum and samples in the appropriate well respectively.
4. **Incubation:** Seal the plate with a cover and incubate at 37℃ for 60 min in water bath.
5. **Washing:** After incubation, remove the cover and wash plate 5 times with wash buffer. Soak for 30-60 seconds. After the 5th washing, remove all wash buffers through aspiration or pouring.
6. **Add HRP-Conjugates/HRP-SA:** Add 100ul of HRP- Conjugates antigen/HRP-SA solution into the above wells (controls, test sample).
7. **Incubation:** Seal the plate with a cover and incubate at 37℃ for 30 min in water bath.
8. **Washing:** After incubation, remove the cover and wash plate 5 times with wash buffer. Soak for 30-60 seconds. After the 5th washing, remove all wash buffers through aspiration or pouring.
9. **Staining:** Add 50ul of TMB substrate A and B into each well respectively. Gently vibrate the plate to mix thoroughly. Cover the plate and incubate at 37℃ for 30 min in water bath.
10. **Stopping:** Add 50ul of stop solution into each well. Gently vibrate the plate to mix thoroughly.

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11. **OD Measurement:** Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution.

Data Analysis

1. **Negative Control:** Under normal conditions, OD values of negative control wells is less than 0.1. (e.g. OD value higher than 0.10 for one negative control well should be discarded. If OD value higher than 0.10 for 2 wells or above, the assay should be repeated.)
2. **Positive Control:** Under normal conditions, OD values of positive control wells is higher than 0.8.
3. **Calculation of Cutoff Value(C.0.):** Cutoff Value = NCx +0.1(NCx less than 0.05 is calculated as 0.05.)
4. **Calculation of Results:**

   Samples with S/C.0.≥1: The reaction of HIV antibody or HIV-1 p24 antigen is positive.

   Samples with S/C.0.<1: The reaction of HIV antibody and HIV-1 p24 antigen is negative.

Assay Limitations

1. Any assay can't absolutely ensure antibodies or antigens with low concentration are not included in samples. Thus, negative result can't exclude the possibility that HIV has been exposed and infected.
2. This kit is only suitable for detecting individual serum or plasma sample. The detection result for other body fluids and samples may be inaccurate.
3. The detection result can't distinguish infections from different subtypes of HIV.

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