Technical Manual

Human HIV-1/2 Antibody ELISA Kit

- **Catalogue Code: HDES0034**
- **Antibody ELISA Kit**
- **Research Use Only**

1. Test principle

This ELISA kit uses the Sandwich-ELISA as the method to detect the HIV I and II antibody in human serum, plasma. The ELISA Microtiter plate provided in this kit has been pre-coated with recombinant HIV I and II antigen. Samples are added to the ELISA Microtiter plate wells and the HIV I and II antibody in which will combine with the pre-coated antigens to form antigen-antibody compound. Free components are washed away. The HRP conjugated HIV I and II antigens are added to each well and react with the compound to form “HIV antigen-HIV antibody-HRP conjugate” compound. The substrate reagent is added to initiate the color developing reaction. The presence of HIV I and II antibody can be determined according to the OD value by using a microplate reader with 450 nm (630 nm) wavelength.

2. Kit components

Item	Specification
ELISA Microtiter plate	96 wells
HRP Conjugate	6 mL
20×Concentrated Wash Buffer	50 mL
Substrate Reagent A	6 mL
Substrate Reagent B	6 mL
Stop Solution	6 mL
HIV-1 Positive Control	1 mL
HIV-2 Positive Control	1 mL
Negative Control	1 mL
Sealed Bag	1 piece
Plate Sealer	3 pieces
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

3. Other materials required but not supplied

- Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm).
- High-precision transferpettor, EP tubes and disposable pipette tips.
- 37° C Incubator or water bath.
- Deionized water.
- Absorbent paper.

4. Notes

1. Please read the manual carefully before use, changes of operation may result in unreliable results.
2. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.
3. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
4. The ELISA Microtiter plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
5. Concentrated washing liquid at low temperature condition is easy to crystallization, it should be adjusted to room temperature in order to dissolve completely before use.
6. The results shall depend on the readings of the Microplate Reader.
7. **Each reagent is optimized for use in the HDES0034. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0034 with different lot numbers.**
8. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

Storage and expiry date

Store unopened at 2 to 8° C. Do not freeze.

Please store the opened plate at 2 to 8° C, the shelf life of the opened kit is up to 1 month.

Expiry date: expiration date is on the box.

5. Sample preparation

1. **Serum/plasma**: Human serum and plasma can be used as detected sample. Fresh collected samples should be fully centrifuged, then take clear liquid for test. Suspended fibrous protein may cause a false positive. Obviously contaminated samples can't be detected.
2. Anticoagulant (EDTA, sodium citrate and heparin sodium) in samples do not affect the result of the experiment in general. Endogenous interference substances in serum such as blood fat, cholerythrin, and hemoglobin will not affect the results. Positive samples like HCV, HBV and RF may not affect the results in general.
3. There should be no microorganism contained in the samples. Samples can be stored at 2-8° C for one week. If samples not tested in a week, store them at below -20° C and avoid freeze-thaw cycles.
4. **Wash Buffer**: The **20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, and then dilute it with deionized water at 1:19.

6. Assay procedure

Restore all reagents and samples to room temperature (25°C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8° C.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. Set 1 well for blank control, 3 wells for negative control and 2 well for positive control. **Samples need test in duplicate.**
2. **Add sample:**
   a) Add 50 μL of control serum respectively to 3 wells for negative control, 1 well for HIV-1 positive control and 1 well for HIV-2 positive control, keep the blank control well empty. (Blank well is not necessary for dual-wavelength detection).
   b) Add 50 μL of **serum/plasma** into each other wells.
3. **Incubate:** cover the ELISA Microtiter plate with sealer. Incubate for 60 min at 37° C in shading light.
4. **Wash:** remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with **Wash Buffer** and immerse for 30-60s each time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
5. **HRP conjugate:** add 50 μL of **HRP Conjugate** to each well except the blank control well, mix fully.
6. **Incubate:** cover the ELISA Microtiter plate with sealer. Incubate for 30 minutes at 37°C in shading light.
7. **Wash:** repeat step 4.
8. **Add Substrate:** add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** to each well. Gently tap the plate to mix thoroughly. Cover with a new plate sealer. Incubate for 30 minutes at 37°C in shading light.
9. **Stop reaction:** add 50 μL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
10. **OD Measurement:** set the Microplate Reader wavelength at 450 nm (it is recommended to set the dual wavelength at 450 nm/630 nm) to detect A value of each well. Blank well is not essential when using dual wavelength 450 nm/630 nm for detection. **Note: Read the results within 30 min.**

7. Reference value

Normally, blank well (just **s**ubstrate agent and stop solution) absorbance: A450 ≤ 0.08;
positive control (PC): A_{450} > 0.80 and average A value of negative control (NC): A_{450} < 0.08.

8. Interpretation of test results

Use each test result independently. Determine the result according to the Cut Off value.
Cut Off(C.0) = 0.10 + average A value of negative control(NC) (when average A_{450} of NC < 0.05, calculate at 0.05; while average A_{450} of NC ≥ 0.05, calculate at the actual value).

1. Positive result: average A value of sample ≥ Cut Off.
2. Negative result: average A value of sample < Cut Off.

9. Limitations of test method

1. This test is only used as the qualitative detection of HIV antibodies in serum and plasma of human.
2. The detection results of this kit are only for reference. For confirmation of the result, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for result.

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Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com