Human Immunodeficiency Virus (HIV-1/2) Antibodies ELISA Kit
Catalog No: MBS2548685
96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.
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 conjugated” compound. The TMB substrate is added to initiate the color developing reaction. The presence of HIV I and II antibody can be determined according to the OD value after colorimetric assay with the Microplate Reader.

Kit components

<table>
<thead>
<tr>
<th>Specification</th>
<th>96 T</th>
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</thead>
<tbody>
<tr>
<td>ELISA Microtiter plate</td>
<td>96 wells</td>
</tr>
<tr>
<td>HIV-1 Positive Control</td>
<td>1 mL</td>
</tr>
<tr>
<td>HIV-2 Positive Control</td>
<td>1 mL</td>
</tr>
<tr>
<td>Negative Control</td>
<td>1 mL</td>
</tr>
<tr>
<td>HRP Conjugate</td>
<td>6 mL</td>
</tr>
<tr>
<td>20×Concentrated Wash Buffer</td>
<td>50 mL</td>
</tr>
<tr>
<td>Substrate Reagent A</td>
<td>6 mL</td>
</tr>
<tr>
<td>Substrate Reagent B</td>
<td>6 mL</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>6 mL</td>
</tr>
<tr>
<td>Sealed Bag</td>
<td>1 copy</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td>3 pieces</td>
</tr>
<tr>
<td>Manual</td>
<td>1 copy</td>
</tr>
</tbody>
</table>

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

Experimental instrument
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
Sample preparation

1. 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.

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, cholesteryl, and hemoglobin will not affect the results. Positive samples like HCV, HEV and RF may not affect the results in general.

3. Samples can be stored at 2~8°C for one week and stored at -20°C for more than a week. Avoid freeze-thaw cycles. Freezing samples should be mixed fully before test.

4. Wash Buffer: Dilute the 20×Concentrated Wash Buffer for 20 times with deionized water.

Assay procedure

Restore all reagents and samples to room temperature 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. Add sample:
   a) Take out Microtiter plate and mark it, reserve 1 well for blank control (empty), 3 wells for negative control, 1 well for HIV-1 positive control and 1 well for HIV-2 positive control. Add 50 μL of negative control/positive control to negative control/positive control wells, keep the blank control well empty. (Blank well is not necessary for dual-wavelength detection)
   b) Add 50 μL of serum sample to other sample wells.
   c) Gently tap the plate to mix thoroughly.

2. Incubate: cover the ELISA Microtiter plate with plate sealer. Incubate for 60 minutes at 37°C in shading light.

3. Wash: after incubation, 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).

4. HRP conjugate: add 50 μL of HRP Conjugate to each well except the blank control well.

5. Incubate: cover the ELISA Microtiter plate with sealer. Incubate for 30 minutes at 37°C in shading light.


7. 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.

8. Stop reaction: add 50 μL of Stop Solution to each well, gently tap the plate to mix thoroughly.

9. 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.
Reference value

1. Result analysis
   (1) Use each test result independently. Determine the result according to the Cut Off value.
   (2) Calculate the Cut Off: \( \text{Cut Off}(C.0) = 0.10 + \text{average } A \text{ value of negative control (NC)} \) (when average \( A_{450} \) of NC \(<0.05\), calculate at 0.05; while average \( A_{450} \) of NC \(\geq 0.05\), calculate at the actual value).

2. Quality control
   (1) Blank well (just chromogenic agent and stop solution) absorbance \(\leq 0.08\).
   (2) Positive control (PC): average \( A_{450} \geq 0.80\).
   (3) Negative control (NC): average \( A_{450} < 0.08\).
   The experimental result is valid if quality control is valid.

3. Determination of results
   (1) Positive result: Sample absorbance \(\geq\) Cut Off.
   (2) Negative result: Sample absorbance \(<\) Cut Off.

Interpretation of results
1. Negative result indicates there is no HIV I / HIV II antibodies detected in samples, while positive result means the opposite.

Limitations of test method
1. The detection results of this kit are only for reference. For confirmation of the diagnosis, please combine the symptoms and other methods of detection, this detection cannot be used as the only criteria for diagnosis.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
Notes
1. This kit is for research use only. It is disposable and cannot be used repeatedly.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. 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.
4. 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.
5. 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.
6. 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.
7. The results shall depend on the readings of the micro-plate Reader.
8. Do not use components from different batches of kit.
9. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.

Storage and shelf life
Store unopened at 2 to 8°C for 6 months. Do not freeze.
Please store the opened kit at 2~8°C, protect from light and moisture. The shelf life of the opened kit is up to 1 months.
Expiry date: expiration date is on the box.