Bio.Pro

Human Immunodeficiency Virus (HIV) Antibody/Antigen ELISA Test Kit (Serum/Plasma)

Package Insert

For in vitro diagnostic and professional use only.

PRODUCT NAME

Human Immunodeficiency Virus (HIV) Antibody /Antigen ELISA Test Kit (Serum/Plasma)

INTENDED USE

This kit is intended for the qualitative detection of HIV-1 and HIV-2 antibodies and P24 antigen in human serum/plasma, which is based on Enzyme Linked Immunosorbent method. The kit is suitable for clinical screening and diagnosis of Human Immunodeficiency Virus infection.

TEST PRINCIPLE

The kit is designed based on enzyme-linked immunosorbent assay with double antigen sandwich method and double antibody sandwich method.

HIV antigen and P24 monoclonal antibody is absorbed in solid phase to the polystyrene reaction microplate, work with biotin antibody, enzyme labeled antigen, enzyme labeled affinity and chromogenic reagent and other reagents to detect HIV-1 and HIV-2 antibody and P24 antigen in human serum/plasma.

COMPONENTS

Materials provided with the kit:

Components	96T/Box		480T/Box	
Coated Plate	1 bag	96 wells	5 bags	96 wells
Enzyme Conjugate	1 vial	12 mL	5 vials	12 mL
Biotin Working Liquid	1 vial	8 mL	5 vials	8 mL
Wash Buffer (40X)	1 vial	20 mL	5 vials	20 mL
Substrate A	1 vial	7 mL	5 vials	7 mL
Substrate B	1 vial	7 mL	5 vials	7 mL
Stop Solution	1 vial	6 mL	5 vials	6 mL
Negative Control	1 vial	1 mL	5 vials	1 mL
HIV-1 Antibody Positive Control	1 vial	1 mL	5 vials	1 mL
HIV-2 Antibody Positive Control	1 vial	1 mL	5 vials	1 mL
HIV-P24 Antigen Positive Control	1 vial	1 mL	5 vials	1 mL
Closure Plate Membrane	3 sheets		15 sheets	

Note: different batches of reagent kit, and different components cannot be exchanged for use. Once open, stable for 3 months at 2-8°C.

SAMPLE REQUIREMENTS

1. **Specimen Collection:** No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum/plasma specimens can be used with this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible to avoid hemolysis of the RBC. Care should be taken to ensure that the serum/plasma specimens are clear and not contaminated by microorganisms.

2. ***Highly lipaemic, icteric, or hemolytic specimens should not be used*** as they can give false results in the assay. ***Do not heat inactivate specimens***. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.

3. HIV ELISA is intended ONLY for testing of individual serum/plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

4. Transportation and Storage: Store specimens at 2-8°C. Specimens not required for assaying within 3 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

TEST PROCEDURE

1. All reagents should be allowed to reach room temperature for at least 15 minutes before use.

2. Dilute the wash buffer at the rate of 1:40 dilution with distilled water before use.

3. The sample should be corresponding to the number of microplate, each plate should be provided with negative control 3 wells, positive control 3 wells and blank control 1 well. (If detect with dual wavelength detection, setting no blank control well is allowed).

Note: Use a separate disposal pipette tip for each specimen, Negative and Positive Control to avoid cross contamination.

4. Add 20μL biotin working liquid in the corresponding well (Do not add in the blank well).

5. Add 100μL sample in the corresponding well, add 100μL negative control and positive control to negative control wells and positive control wells (Do not add in the blank well).

6. Shake for 30 seconds with an oscillator (This step is very important). Incubate at 37°C for 60 minutes with the sealing plate membrane sealing the plate.

7. At the end of the incubation, remove and discard the plate cover. Take out, add wash buffer to each well for 20 seconds. Repeat 5 times. After the final washing cycle, turn the plate over onto blotting paper or clean towel, and tap it to remove any remainders.

8. Add 100μL Enzyme Conjugate in the corresponding well (Do not add in the blank well). Mix gently by shaking.

9. Incubate at 37°C for 30 minutes with the sealing plate membrane sealing the plate.

10. Repeat the wash step for 5 times as in step 7.

11. Add Substrate A (50μL) and Substrate B (50μL) (Do not add in the blank well). Mix gently by shaking. Incubate at 37°C for 30 minutes with the sealing plate membrane sealing the plate.

12. Add 50μL Stop Solution to each well (Do not add in the blank well). Mix gently by shaking, read the absorbance within 10 minutes after stopping the reaction. Calibrate the plate reader with the Blank well and read the absorbance at 450nm.If a dual filter instrument is used, set the reference wavelength at 630nm. Set no blank wells is allowed if use dual wavelength to detect. Calculate the Cut-off value and evaluate the results.

INTERPRETATION OF RESULTS

Colorimetry: Read the sample's optical density (OD) at 450nm with a microplate reader.

Mean negative control OD value ≤ 0.1 and positive control OD value ≥ 0.8, the test is valid, otherwise the test is invalid.

Cut-Off value (C.O.) = Mean negative control OD value + 0.12

Positive Results: Sample OD value/C.O. ≥ 1.0

Specimens giving an absorbance equal to or greater than the Cut-Off value is considered initially reactive, which indicates that HIV-1 and HIV-2 antibody and P24 antigen has probably been detected using HIV ELISA. All initially reactive specimens should be retested in duplicates using HIV ELISA

before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for HIV with HIV ELISA.

Negative Results: Sample OD value/C.O. < 1.0

Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no HIV-1 and HIV-2 antibody and P24 antigen has been detected with HIV ELISA, therefore the patient is probably not infected with HIV and the blood unit do not contain HIV and could be transfused in case that other infectious diseases markers are also absent.

Borderline: Sample OD value/C.O.= 0.9-1.1

Specimens with OD value to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicate is required to confirm the results.

Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system (e.g. PCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

LIMITATIONS

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. The reagent is a qualitative reagent, and cannot be used as a quantitative reagent.
3. This reagent is only used for the detection of human serum/plasma samples.

PERFORMANCE CHARACTERISTICS

1. Test of HIV antibody:
   1.1 Negative Specificity: The coincidence rate should exceed 18/20 when detecting negative quality control samples of anti-HIV 1/2.
   1.2 Positive Specificity: When detecting positive quality control samples of anti-HIV 1/2 the HIV-1 positive reference conformity rate (+/+) should be 18/18; The coincidence rate of HIV-2 positive reference material (+/+) should be 2/2.
   1.3 Limit of Detection: At least 3/6 results should be positive when detecting HIV limit quality control samples of anti-HIV 1/2, the matrix fluid(S1) should result in negative.
   1.4. Precision: Intra-assay: CV%≤15%; Precision between batches: CV%≤20%.
2. Test of HIV-1 P24 antigen:
   2.1 Negative Specificity: The coincidence rate should exceed 20/20 when detecting negative quality control samples of HIV-1 antigen p24.
   2.2 Positive Specificity: The coincidence rate should be 10/10 when detecting positive quality control samples of HIV-1 antigen p24.
   2.3 Limit of Detection: L4 should be positive when detecting HIV limit quality control samples of HIV-1 antigen p24, matrix serum (L10) should be negative.
   2.4. Precision: Intra-assay: CV%≤15%; Precision between batches: CV%≤20%.

ATTENTIONS

1. Do not exchange reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. Allow the reagents and specimens to reach room temperature before use. Shake reagent gently before use.
4. Concentrated washing liquid will produce crystal at room temperature, should be diluted completely before use.
5. Please put the unused plate back into the bag, and store at 2-8°C.
6. Operate strictly according to the instruction, control of reaction time and temperature strictly.
7. Never reuse microplate sealing membrane. If the external of the microplate contact with water when warm bath, results will be better.
8. Use sufficient volume of washing liquid in the washing steps. Fail to do so may cause color deepening.
9. Negative results of reagent do not rule out the possibility of HIV infection. Positive results must be combined with clinical information for analysis.
10. All the reagents were treated by inactivation, but still should be regarded as potentially infectious. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.

STORAGE AND EXPIRY

1. Store at 2-8°C.DO NOT FREEZE. Valid for 18 months.
2. Once open, stable for 3 months at 2-8°C. Other liquid components have the same validity period with the reagent box.

BIBLIOGRAPHY

[1] Pharmacopoeia of the People's Republic of China.
[2] Requirements for Biologics of the People's Republic of China.

INSTRUCTIONS OF SYMBOL

	Consult instructions for use		Keep dry
	Temperature limit		Batch code
	Do not re-use		In vitro diagnostic medical device
	Manufacturer		Date of manufacture
	Use-by date		Contains sufficient for <n> tests
	Keep away from sunlight		

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