

Antibody to Human Immunodeficiency Virus (HIV) EIA

This anti-HIV 1/2 EIA Test Kit is a qualitative detection of antibodies to HIV-1 and HIV-2 in human serum/plasma.

REF: BS.1/HIV01.096.0096

96 test

CLINICAL SIGNIFICANCE

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS). Infection with HIV leads to progressive deterioration of the immune system, increasing susceptibility to opportunistic infections and malignancies. Early detection of antibodies to HIV-1 and HIV-2 is essential for diagnosis, prevention of transmission, blood screening, and patient management. This assay is intended for the qualitative detection of antibodies to HIV-1 and HIV-2 in human serum or plasma, and may be used as an aid in the diagnosis of HIV infection and in screening of blood donors.

METHOD PRINCIPLE

The kit uses a double-antigen sandwich assay to detect human immunodeficiency virus (HIV) antibodies (type 1+2). The microplate are pre-coated with human immunodeficiency virus recombinant antigen (gp36, gp41, gp120). During the testing, if the samples contain antibodies to HIV, it will combine with the coated human immunodeficiency virus recombinant antigen, wash the free components off, and the horseradish peroxidase-labeled human immunodeficiency virus recombinant antigen is added to form a double-antigen complex and washed to remove the free components, add the substrate, read the absorbance value with a microplate reader and judge the result.

COMPONENTS

Materials provided with the kit: (Components 96T/Box)

Components	96 T/Box	
Coated Plate	1 bag	96 wells
Enzyme Conjugate	1 vial	6.0 mL
Wash Buffer (40X)	1 vial	50 mL
Substrate	1 vial	12 mL
Stop Solution	1 vial	6.0 mL
Negative Control	1 vial	6.0 mL
HIV-1 Antibody Positive Control	1 vial	1.0 ml
HIV-2 Antibody Positive Control	1 vial	1.0 ml
Negative Control	1 vial	1.0 ml

Note: different batches of reagent kit, and different components cannot be exchanged for use.

PRECAUTIONS AND WARNINGS

Good Laboratories practices using appropriate precautions should be followed in:

Wearing personnel protective equipment (overall, gloves,

Glasses. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries, seek medical advice immediately.

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. Failure to do so may cause color deepening.

STORAGE AND EXPIRY

Store at 2-8°C. DO NOT FREEZE. Valid for 12 months. Once open, stable for 1 months at 2-8°C. Other liquid components have the same validity period with the reagent box.

SPECIMEN COLLECTION AND PRESERVATION

1. 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 must be separated from the clot as early as possible to avoid hemolysis of the RBC. Care should be taken to ensure that the 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 EIA 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.

Procedure

1. All reagents should be allowed to reach room temperature for 15 minutes before use.
2. Dilute the wash buffer at the rate of 1:40 dilution with distilled water before use.
3. Samples should correspond to the quantity of microplates, and each microplate should be provided with three negative control wells and two positive control wells. (each 1 for HIV-1 and HIV-2) and blank control 1 well. (No sample and conjugate are needed in blank control well other steps are the same as all the wells. 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 100 µL of the sample in the corresponding well, 100 µL negative control and positive control respectively to the corresponding wells. (Do not add in the blank well, Do not add HIV-2 positive control if there is no need in the experiment).
5. Shake for 30 seconds with an oscillator. A 37°C constant temperature water bath for 60 minutes with the sealing plate membrane sealing the plate.
6. At the end of the incubation, remove and discard the plate cover. Take out add wash buffer to each well for 20 seconds. Repeat wash for 5 times. After the final washing cycle, turn the plate over onto blotting paper or clean towel and tap it to remove any remainders.
7. Add 100 µL of the enzyme conjugate to each well. (Do not add in the blank well). Mix gently by shaking.
8. Incubate in 37 C for 30 minutes with the sealing plate membrane sealing the plate.
9. Repeat the wash step for 5 times as in steps 6.
10. Add 50 µL (1 drop) of Substrate A and 50 µL (1 drop) of Substrate B. Shake gently to mix. React at room temperature and away from light for 20 minutes.
11. Add 50 µL (1 drop) of stop solution to each well. Gently shake to mix and read. Absorbance within 10 minutes after the reaction stops. Read the absorbance at 450 nm. If a dual-filter instrument is used, please set the reference value wavelength to 630 nanometers. Calculate the cutoff value and evaluate the result.

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact the technical support.

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. **Negative Specificity:** The coincidence rate should exceed

18/20 when detecting negative quality control samples with EIA kits of anti-HIV 1/2.

2. Positive Specificity: When detecting positive quality control samples with EIA kits of anti-HIV 1/2 the HIV-1 positive reference conformity rate (+/+) should be 18/18; P12 must be ≥P11; The coincidence rate of HIV-2 positive reference material (+/+) should be 2/2.

3. Limit of Detection: At least 3/6 results should be positive when detecting HIV limit quality control samples with the EIA kits of anti-HIV 1/2, the matrix fluid(S1) should result in negative.

4. Precision: Intra-assay: CV% ≤ 15%; Precision between batches: CV% ≤ 20%.

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 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.

Specimens giving an absorbance equal to or greater than the Cut-Off value are considered initially reactive, which indicates that HIV-1 and HIV-2 antibody has probably been detected using HIV EIA. All initially reactive specimens should be retested in duplicates using HIV EIA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for HIV with HIV EIA

Negative Results: Sample OD value < C.O.

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

REFERENCES

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

SYMBOLS IN PRODUCT LABELLING		
	For in-vitro diagnostic use	 Number of <n> test in the pack
	Batch Code/Lot number	 Caution
	Catalogue Number	 Do not use if package is damaged
	Temperature Limitation	 Consult Instruction for use
	Expiration Date	
	Manufactured by	

