HIV 1,2 Antibody ELISA Kit

96 Tests Kit
Enzyme Immunoassay for the Detection of Human Immunodeficiency Virus type 1 and 2 Antibody
(For In Vitro Diagnostic Use Only)
Catalogue No. PT-HIV1,2-96

PISHTAZ TEB DIAGNOSTICS

Introduction

Human Immunodeficiency Virus (HIV) is a member of Retroviridae family which is subdivided into two types of HIV-1 and HIV-2. HIV-1, the most prevalent type with world-wide distribution has been introduced by Luc Muntanier at 1983 in French and its full characteristics were described by Robert Galo and Jay Levy in US. Three years after detection of first HIV type the second type; HIV-2 has been described in West-African region at 1986. The viral genome consists of single-stranded RNA with the size of 9.7 Kb. This virus is etiologic agent of Acquired Immunodeficiency Syndrome (AIDS) with the latent period of about 8 years since entrance of virus into human body. During this latent period, the viruses proliferate and destroy lymphocytes which involved in cellular immune response and leads to fatal opportunistic fungal, viral and parasitic infections.
To diagnose infected patients, detection of specific antibody against HIV viral antigens is widely used. Among various methods which are exploited for the detection of HIV antibody, ELISA test offers reliability and high sensitivity particularly in preliminary and screening diagnosis. Positive samples should be rechecked and confirmed by Western blot analysis.
This kit has third generation HIV ELISA format with high sensitivity and specificity and detects Antibody against both HIV-1 and HIV-2 types.

Test Principle

The test principle is based on antigen sandwich ELISA immunoassay. In this technique to create first component of sandwich, micro wells are coated with certain amount of HIV-1 recombinant antigens called p24, gp41, gp120 and gp36 of HIV-2 type. Then serum sample are allowed to react with solid phase antigens. If specific antibodies (IgG, IgM, IgA) are presented in the serum they will bind to HIV antigens through their individual Fab section. After incubation at 37°C, the wells are washed to remove unbound antibodies. To create second sandwich component, HIV-1 and HIV-2 recombinant antigens which are conjugated with HRP are added into the wells following another incubation and wash step. A solution of chromogen-substrate is added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of specific anti–HIV is directly proportional to the color intensity of the test sample.

Materials Provided With Kit

1. Antigen coated plate (1 plate, 96 wells): Microtiter wells coated with HIV1,2 recombinant antigens.
2. Sample diluent (1 vial, 15 ml) : Contains Phosphate buffer solution containing protein as stabilizer and Kathon CG as preservative.
3. Enzyme Conjugate (1 vial, 1 ml): HIV1,2 recombinant antigens labeled with HRP (20X). Should be diluted 1:20 with conjugate diluent.
4. Conjugate diluent (1 vial, 15 ml) : Contains Phosphate buffer solution containing protein as stabilizer and Kathon CG as preservative.
5. Positive control (1 vial, 1 ml): Contains inactivated human serum containing anti-HIV-1 & Anti HIV-2 antibodies with 0.05% Kathon CG as preservative.
6. Negative control (1 vial, 1 ml): Contains inactivated human serum without antibody against HIV types and contains 0.05% Kathon CG as preservative.
7. Wash solution (1 vial, 50 ml): Contains phosphate buffer saline (PBS) containing 0.05 % Tween 20 as detergent and Kathon CG as preservative, concentrated (20X).
8. Chromogen-substrate (1 vial, 12 ml): Contains tetra-methyl benzidine and hydrogen peroxide., ready to use.

9. Stop solution (1 vial, 12 ml): 1 molar hydrochloric acid solution.
10. Cardboard sealer.

Materials/Equipment required but not provided with Test Kit

1. ELISA reader with 450 and 630 nm (reference filter).
2. Precision pipettes 50 and 100 µl.
3. 37°C water bath or incubator.
4. 5% sodium hypochlorite solution.
5. Disposable pipette tips.
6. Absorbent paper.

General Information

1. Do not mix kit reagents from different lot numbers. All kit components must be used only in original kit.
2. This kit is just for the detection of Anti-HIV antibody in human serum and plasma.
3. All reagents obtained from human sources are negative for HBs Ag, HCV Ab and HIVAg. The positive control contains HIV1,2 antibodies, and is inactivated by incubation at 56°C for 30 minutes. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.
4. Positive sera, wash solution residuals and equipments suspected to contaminated by HIV, should be disinfected by 5% hypochlorite soultion for 30 minutes or autoclaved at 121°C for 60 minutes.

Storage Conditions

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants. Opened kits are stable for 4 months.
3. Reagents stability is marked on their labels. Please ensure that do not use expired date reagents.

Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. Do not use diluted or pooled sera/plasmas. If testing cannot be done within an hour after sample collection, refrigerate the specimen (maximum 1 week at 2-8°C) and let it return to room temperature before testing. If prolong storage is required, samples should be stored at -20°C. Avoid freeze-thaw of specimen during storage. Samples suspected to microbial contamination should not be used.

Reagents & Specimens Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: dilute concentrated wash solution 1:20 with distilled water before use.
3. Test steps should be done sequentially.
4. Working enzyme conjugate: Dilute the concentrated conjugate with the con-jugate diluent (1:20) according to table 1. Mix it gently. This working solution remains stable for 4 weeks at 2-8°C.

TABLE 1

Strips required	1	2	3	4	5	6
Conjugate diluent ml	0.95	1.9	2.85	3.8	4.75	5.7
Concentrated conjugate ml	0.05	0.1	0.15	0.2	0.25	0.3
Strips required	7	8	9	10	11	12
Conjugate diluent ml	6.65	7.6	8.55	9.5	10.45	11.4
Concentrated conjugate ml	0.35	0.4	0.45	0.5	0.55	0.6

Assay Procedure

1. Use required number of wells and keep the remaining with desiccants in tightly closed sealed bag. Consider first well as **Blank** and next two wells for **Positive** and **Negative controls**.
2. Add 100 µl of sample diluent into each well except blank.

3. Add 50 µl of positive and negative controls as well as test sera into appropriate wells.
4. Seal the plate with cardboard sealer tightly. Mix gently for 15 seconds. Leave wells for 60 minutes at 37°C. (water bath/incubator)
5. Remove the wells content by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times (each with 300 µl of Working wash solution).
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Add 100 µl of working enzyme conjugate into each well except blank.
8. Cover the plate with cardboard sealer tightly. Leave wells for 30 minutes at 37°C.
9. Repeat step 5 and 6.
10. Dispense 100 µl of chromogen/ substrate to each well.
11. Incubate the microplate wells at room temperature and dark for 15 minutes to develop color.
12. Add 100 µl of stop solution to the wells to stop reaction.
13. Read absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available) reference filter is highly recommended.

Validity of the Assay

The assay is to be considered valid if:

1. The OD 450 nm of the blank well is lower than 0.1. Higher values indicate chromogen/substrate contamination. In such a case, repeat the assay carefully and check the reagent.
2. The OD value for the negative control is lower than 0.15. Higher values indicate an incorrect washing procedure. In such a case, check the efficiency of the washing device.
3. The mean OD value of positive control is higher than the 1.2. Lower values indicate kit reagents decay. In such a case, check expiry date of the kit before repeating the assay.

Result Calculation

1. Measure absorbance of controls and samples at 450 nm (Use 630 nm filter as reference filter if it's available).
2. To calculate cut off value for the test, use following formulae:

Cut-off = Negative control mean OD (450 nm) + 0.15

3. Negative results indicate absence of or undetectable antibody response against HIV antigens in early stages of infection.
4. Positive results should be rechecked and those which become negative should be reported as negative.
5. Faulty washing and sampling errors may leads to positive results.
6. In case of rechecked with positive results, confirmatory tests like Western blot analysis or PCR should be done.

Performance Characteristics

- Sensitivity

To evaluate test sensitivity, BBI panels were used. They include:

1. Sero-conversion panels

Results with these panels are shown in table 2.

TABLE 2

No.	Panel name	First positive sample with HIV1,2 Ab kit	Days after sampling that test become positive
1	Panel J (PRB910)	3	26
2	Panel L (PBR912)	2	9
3	Panel N (PRB914)	1	0
4	Panel Q (PRB917M)	5	65
5	Panel V (PRB922)	1	0
6	Panel AB (PRB927)	2	28
7	Panel Z (PRB928)	5	27
8	Panel AF (PRB931)	6	28
9	Panel AT (PRB944)	5	14

2. Anti HIV-1,2 Combo Performance (PRZ206) Panel

This panel contains 13 members, among them 5 developed positive for Anti-HIV2, 6 became positive for Anti-HIV1 antibody and remaining two members which are negative in panel became negative . All results were also conform with this kit.

3. Anti HIV mixed titer performance (PRB204) panel

This panel includes 23 positive and 2 negative members which were detected by this kit precisely.

This kit is also evaluated by 148 positive samples which were confirmed by Western blot analysis and the test results displayed 100% sensitivity which is comparable to other commercial tests.

- Specificity

To evaluate test specificity, 2460 random serum/plasma samples were tested with this kit. Results showed 3 positive samples which on re-check 2 samples remained positive and one became negative. The estimated specificity of kit is 99.5-100%.

- Interferences

Results obtained from known serum samples with HCV, ANA and RF antibodies as well as hemolytic and icteric samples are shown in table 3.

TABLE 3

Serum type	No. of samples tested	Result
ANA positive	14	Negative
Anti-HCV Positive	20	Negative
RF Positive	15	Negative
Hemolytic Serum	8	Negative
Lipemic Serum	7	Negative

- Precision

Within and Between-run precision were performed on positive and negative serum controls and also on three positive serums. Results are shown in table 4 and 5.

TABLE 4. WITHIN ASSAY PRECISION

Serum type	Test repeat	Mean OD	SD	CV%
Positive control	18	2	0.15	6.7
Negative control	18	0.044	0.005	11.2
Positive serum 1	18	1.4	0.07	5.3
Positive serum 2	18	0.96	0.09	9.5
Positive serum 3	18	0.45	0.05	11.4

TABLE 5. BETWEEN ASSAY PRECISION

Serum type	Test repeat	Mean OD	SD	CV%
Positive control	10	1.94	0.05	2.6
Negative control	10	0.046	0.007	15.8
Positive serum 1	10	1.49	0.14	9.9
Positive serum 2	10	0.92	0.09	9.9
Positive serum 3	10	0.46	0.05	11.2

All samples were run in duplicate.

References

1- Guidelines for the use of antiretroviral agents in pediatric HIV infection.Center for Disease Control and Prevention , 1998; 47; No.RR-4.

2-Barbe, F.et al., (1994) Early detection of anti bodies to HIV-1 by a third generation enzyme immunoassay. Ann. Biol. Clin. (Paris), 52: 341-345.

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4-Barbe, F.et al.,(1994) Early detection of antibodies to HIV-1 by a third geneartion enzyme immunoassay.Ann.Biol.Clin.(Paris) 52: 341-345

5-Clavel F.et al., (1987)Human immunodeficiency virus type 2 infection associated with AIDS in west Africa New Eng.J.Med.316(19):1180-1185)

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of west-central Africa origin , J.Virol.64, 1207-1216
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HIV ELISA Test Procedure

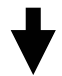
Step 1

Reagent	Blank	Control Serum	Sample
Sample Diluent	None	100 µl	100 µl
Control Serum (P/N)	None	50 µl	None
Sample	None	None	50 µl

Cover the microplate wells with cardboard sealer tightly, Mix gently for 15 seconds and incubate them for 60 minutes at 37°C.

Step 2

Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.



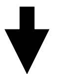
Step 3

Enzyme conjugate	None	100 µl	100 µl

Cover the microplate wells with cardboard sealer and incubate for 30 minutes at 37°C.

Step 4

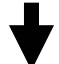
Remove plate cover and discard reagents of wells. Wash the microplate wells for 5 times according to test manual.



Step 5

Chromogen -substrate solution	100 µl	100 µl	100 µl

Incubate wells for 15 minutes at room temperature and dark.



Step 6

Stop Solution	100 µl	100 µl	100 µl

Read absorbance at 450 nm (Use 630 nm filter as reference filter if it’s available).