

ELISA Kit for Antibody to Human Immunodeficiency Virus 1&2 (gp 36 and gp 41)

[NAME AND INTENDED USE]

***ELISA Kit for Antibody to Human Immunodeficiency Virus 1&2 *** is an *in vitro* enzyme immunoassay for the detection of anti-HIV antibodies in human serum or plasma.

[PRINCIPLE]

This kit uses two-antigen sandwich ELISA method to detect anti- HIV (1+2) antibodies in serum or plasma. The HIV antigens (**gp 36 and gp 41**) with high purity are coated on the multi-wells strips. When serum sample and HIVAg labeled with HRP (conjugated) are added to the coated wells, and if Anti-HIV is present in the sample, a complex of HIVAg-Anti-HIV-HIVAg labeled with HRP will form. The enzyme reaction with substrate TMB produces a color change, and the intensity of the absorbance at 450 nm indicates the presence or absence of Anti-HIV antibodies in the sample. The test is specific, sensitive, reproducible and easy to operate. It is for blood screen of HIV infection.

[MATERIALS PROVIDED]

1. HIV Antigen Coated Microwell Plate	1 block (96wells)
2. Sample Diluent	1 bottle (6ml)
3. Enzyme Conjugant	1 bottle (12ml)
4. Negative Control Serum	1 vial (1ml)
5. Positive Control Serum	1 vial (1ml)
6. Concentrated Wash Buffer (1:20 dilution prior to use)	1 bottle (50ml)
7. Substrate A	1 bottle (6ml)
8. Substrate B	1 bottle (6ml)
9. Stop Solution	1 bottle (6ml)
10. Plastic Bag	1 bag
11. Seal Paper	3 pieces
12. Manual	1 each

[SAMPLE COLLECTION AND PRESERVATION]

Blood serum samples are routinely prepared from vein. Blood plasma sample are routinely prepared with routine amount of anticoagulant such as heparin or sodium citrate. Sample can be stored at 4°C if tested within five days. Sample can be stored at -20°C at least for 3 months. Avoid hemolysis and repetitive freeze and thaw of samples. Samples with cloud or precipitation should be centrifugated or filtered before test. Prevent serum from bacteria contamination during collection and storage.

[TEST PROCEDURE]

1. Bring *** ELISA Kit for Antibody to Human Immunodeficiency Virus 1&2 *** (all reagents), and samples to room temperature before use (approximately 30 minutes).
2. Dilute concentrated wash buffer 1:20 with ddH₂O
3. For each test, set one blank, two positive and three negative controls. Add 100 µl positive and negative control serum into positive and negative control wells respectively without sample diluent.
4. Add 50 ul of sample diluent into each test well, add 50 µl test serum into test wells, mix thoroughly,
5. Cover wells with seal paper, incubate for 40 minutes at 37°C.
6. Discard the liquid in all wells and fill the wells with wash solution (300µl per well). Lay aside for 15 seconds, discard the liquid in all wells and fill the wells with wash solution. Repeat 5 times and dry wells after last wash.
7. Add enzyme conjugant 100 µl into the wells except the blank well.
8. Cover wells with seal paper, incubate for 40 minutes at 37°C.
9. Wash plate 5 times as described in Step 6.

10. Add substrate A and B one drop or 50 µl respectively to each well, mix gently, protected from light and lay aside for 15 minutes at 37°C.
11. Add one drop of stop solution (50 ul) into each well to stop the reaction, including blank well.
12. Measure the absorbance at 450nm against the blank, or measure the absorbance at 450nm/630nm.

[INTERPRETATION OF RESULTS]

Colorimetric Method

Cut Off Value calculation:

COV = 0.08 + the average OD of negative controls, (if the average OD of negative controls is below or equal to 0.05, calculate it as 0.05),

Positive OD₄₅₀ of sample ≥ COV

Negative OD₄₅₀ of sample < COV

Positive samples in screen test should be repeated in duplicate. Repeat positive should be confirmed by confirmatory laboratory testing.

[QUALITY CONTROL]

If the OD of positive controls is not below 1.5, OD of negative is not higher than 0.1, the assay result is validated. Otherwise, repeat the test.

[PRECAUTIONS]

1. Only the HIV screen laboratories established under the approval of local sanitation department can use this diagnostic kit.
2. To prevent cross-contamination, wear gloves and working suits throughout the procedure, and execute the disinfection and isolation regulations strictly. Dispose of all samples and materials used to perform the test. The 5.0g/L liquid sodium hypochlorite solution or 121°C high pressure steam may be used to disinfect samples and materials before disposal
3. The samples should be fresh, avoid hemolysis, bacteria growing, and repetitive freeze and thaw.
4. Not for detecting samples from patient injected with globin. Not Recommend for samples with higher lever of lipid, bilirubin or Hemoglobin.
5. Do not interchange reagents between kit lots. The seal paper can't be used repeatedly.
6. Mix reagents well before use. If crystal form in certain reagents, such as wash buffer, it can be used without problems after warm up and mix well.
7. Follow instruction exactly during assay, especially in temperature and time for reactions. All pipetting devices should be used with care and calibrated regularly following the manufacturer's instructions.
8. Put the remained reagents to the sealed pouch, and return to 2~8°C in time.

[STORAGE AND STABILITY]

Store the kit at 2~8°C.

[PERFORMANCE CHARACTERISTICS]

Sensitivity the agreement rate of the tests ≥97.5%

Specificity the agreement rate of the tests ≥97.5%

Precision CV(%) ≤15% (n=10)

[EXPIRATION]

The shelf life is 8 months.

This Kit is for Research Use Only