Human Immunodeficiency Virus (1&2) Elisa test kit

For in-vitro diagnostic use only

2°C 8°C Store at 2 to 8°C

INTENDED USE

This HIV-1,2 ELISA Kit is to be used for the in vitro detection of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2) in serum or plasma

INTRODUCTION

This HIV-1, 2 ELISA Kit employs synthetic HIV polypeptides and recombinant HIV proteins for the detection of antibodies to HIV-1 and HIV-2. These polypeptides and recombinant proteins, which correspond to highly antigenic epitopes consisting of essential sequences derived from both the envelope and core proteins of HIV-1 and HIV-2, constitute the solid-phase antigenic adsorbent. HIV-1,2 ELISA Kit offers a sensitive, reliable and accurate interpretation of the reactivities to HIV with a 60 minute incubation period.

PRINCIPLE OF THE ASSAY

This HIV-1,2 Antibody enzyme linked immunosorbent assay (ELISA) kit employs a technique called a qualitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with synthetic HIV polypeptides and recombinant HIV proteins which correspond to highly antigenic epitopes consisting of essential sequences derived from both the envelope and core proteins of HIV-1 and HIV-2. Samples or controls are added to the microtiter plate wells and incubated. HIV specific antibodies, if present, will bind to and become immobilized by the antigen pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound HIV antibodies and other components of the sample. A standardized preparation of horseradish peroxidase (HRP)-conjugated synthetic HIV polypeptides and recombinant HIV proteins is added to each well to "sandwich" the HIV antibody immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP-conjugate and a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain HIV antibody and enzyme-conjugate will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the colour change is measured spectophotmetrically at a wavelength of 450 nm. Samples with O.D. values greater than or equal to the Cut-off Value are considered reactive by the criteria of this HIV-1,2 Antibody ELISA Kit.

Materials

Materials provided

1. **Coated microplates:**
   One microplate with 96 wells Pre-coated with HIV-1/HIV-2 antigen. Store at 2-8°C.
2. **Enzyme Conjugate:**
   One red cap vial (7.5 ml) containing of HRP (horseradish peroxidase) labeled HIV-1/HIV-2 antigen in Tris-HCl buffer containing proteins of bovine origin. Contains 0.05% ProClin 300® preservative. Store at 2-8°C.
3. **Negative Control:**
   One green cap vial (1.0 ml) of PBS buffer containing proteins of bovine serum. Contains 0.05% ProClin 300® preservative. Store at 2-8°C.
4. **Positive Control:**
   One red cap vial (1.0 ml) of PBS buffer containing heat-inactivated human serum positive for HIV-1/HIV-2 antibody and proteins of bovine serum. Contains 0.05% ProClin 300® preservative. Store at 2-8°C.
5. **Substrate Solution A**
   One blue cap vial (7.5 ml) containing of hydrogen peroxide. Contains 0.05% ProClin 300® preservative. Store at 2-8°C.
6. **Substrate Solution B**
   One brown cap vial (7.5 ml) containing of TMB (3, 3', 5, 5'-tetramethylbenzidine) in a buffer solution. Store at 2-8°C.
7. **Stop Solution**
   One yellow cap vial (7.5 ml) containing of a mixture of sulfuric acid and citric acid. Store at 2-8°C.
8. **Washing solution (20X Conc.)**
   One transparent cap bottle (50 ml) of PBS-Tween at 20 times the strength of the working wash buffer.
9. **Two Plate Sealers.**
10. **One package insert.**
11. **One Zip-lock bag.**

Materials required but not provided

1. Absorbent paper or paper towel.
2. Automated microplate strip washer for washing the microplate.(optional).
3. Distilled or deionized water for diluting the Wash Solution Concentrate.
4. Disposable reagent troughs.
5. Incubator capable of maintaining the temperature limits (37°C±2°C) defined in the assay protocol.
6. Magnetic stirrer for mixing the Wash Solution (optional).
7. Micropipettes and multichannel micropipettes of appropriate volumes. (Recommended: 20µl-200µl and 10µl-50µ l).
8. Microplate reader for reading the absorbance at 450 nm with a reference wavelength of 620 or 630 nm or at a single wavelength of 450 nm.
9. Plate shaker for shaking the plate. (optional).
10. Timer.
11. Vortex mixer for mixing thawed samples. (optional).

PREPARATION OF REAGENT

1. Bring all reagents to room temperature (18-25°C) for at least 30 minutes before use. Mix all reagents prior to use by gentle inversion. Do not induce foaming.
2. Set the incubator to 37 °C.
3. Add 1 volume of Wash Solution Concentrate to 19 volumes of distilled or deionized water to give the required volume, and mix well with a magnetic stirrer. Indicate the batch code, date prepared, and expiry date on the container

PRECAUTIONS

1. Do not substitute reagents from one kit lot to

another. Controls, conjugate, and microtiter plates are matched for optimal performance. Use only reagents supplied by manufacturer.
2. Allow kit reagents to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from storage bag until needed. Unused strips should be stored at 2-8 °C in its pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood erivatives should be considered potentially infectious and good laboratory practices should be followed.
8. All materials should be disposed of in a manner that will inactivate human viruses.
9. Solid Waste: autoclave 60 min. at 121°C.Liquid Waste: add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand a minimum of 30 minutes to
10. Inactivate the viruses before disposal.
11. Substrate B contains 20% acetone, keep reagent away from flaming source.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which mustbe dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION, COLLECTION, HANDLING, AND STORAGE

Serum: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes and serum extracted.

Plasma: Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection there must be quick separation of plasma with less than 30 minutes on ice. Centrifuge for 10 minutes (4°C) to remove any particulates.

1. This HIV-1,2 Antibody ELISA Kit is affected by haemolysis of specimens. No adverse effects have been noted in the presence of anti-coagulants, sodium citrate, EDTA, or heparin.
2. Avoid grossly hemolytic, lipidic or turbid samples
3. Serum or plasma samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
4. When performing the assay slowly bring samples to room temperature.
5. It is recommended that all samples be assayed in duplicate.
6. Do not use heat treated specimens.

STORAGE OF TEST KIT

1. Store all components at 2-8 °C. Do not freeze. Avoid strong light.
2. Place unused wells in the Zip-lock bag with desiccant provided, then seal the zip-lock bag in the aluminium foil pouch with a plate cover and return to 2-8°C, under which conditions the wells will remain stable for 2 months, or until the indicated expiry date, whichever is earlier.
3. Seal and return all the other unused reagents to 2-8°C, under which conditions they will remain stable for 2 months, or until the indicated expiry date, whichever is earlier.

TEST PROCEDURE

1. Bring all reagents and specimens to room temperature (15°C-30°C) before beginning the assay. Swirl gently before use. Adjust the incubator at +37°C, if necessary.
2. Write down the relative numbers of specimens and wells on the data sheet. One well for the blank, five additional wells for the controls and one well for each specimen. If the experiment does not need the whole plate, remove the remaining strips from the strip holder and store the unused strips in the ziplock bag (included in the kit) which contains desiccant.
3. Reserve one well for blank, add 50ul of the Negative Control to each of three wells, 50ul of the Positive Control to each of two wells and 50ul of each specimen to one well according to the following scheme:

1 A	: Blank
2 A, 3 A, 4 A	: Negative Control Serum
5 A, 6 A	: Positive Control Serum
7 A	: Samples

4. Shake on a plate shaker to completely mix the liquids in the wells or agitate manually by gently tapping the sides for 30 seconds.
5. Cover the plate to minimize evaporation, and incubate at 37 °C for 30 minutes.
6. Add 350 µl of wash solution, decant (tap and blot) or aspirate. Repeat 5 additional times for a total of 6 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
7. Add 50 µl of enzyme conjugate to each well except the blank well.
8. Shake on a plate shaker to completely mix the liquids in the wells or agitate manually by gently tapping the sides for 30 seconds. Avoid knocking plates that contain Enzyme Conjugate, to prevent contamination.
9. Cover the plate with a new plate cover to minimize evaporation, and incubate at 37°C for 30 minutes.
10. Add 350 µl of wash solution, decant (tap and blot) or aspirate. Repeat 5 additional times for a total of 6 washes. An automated microplate strip washer can be used. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.
11. Add 50 µl of substrate A, then 50 µl of substrate B to

each well, including the blank well.

12. Gently mix for 15 seconds then cover the plate with a new plate cover to minimized evaporation, and incubate at 37 °C in the dark for 10 minutes.
13. Add 50 µl of stop solution to each well, including the blank well, and mix gently. Complete mixing is required for acceptable results.
14. Read the absorbance within 20 minutes at 450 nm with a reference wavelength of 620 or 630 nm in a microplate reader. If reading the absorbance at a single wavelength of 450 nm, the results should be calculated by subtracting the value of the blank well.

Measurement Results

Each plate must be considered separately when calculating and interpreting results of the assay. Positive and Negative Controls must be run on each plate.

1. Negative Control

Calculate the mean absorbance of the replicates of the negative control.

2. Cut-off Value

Calculate the cut-off value by adding 0.1 to the mean of the negative control replicates (in case the mean absorbance of negative control replicates < 0.05, use 0.05 instead of the actual mean)

3. Example

Negative Control absorbance: well 1 = 0.06, well 2 = 0.01, well 3 = 0.02

Mean Negative Control: (0.06 + 0.01+ 0.02)/3 = 0.03 (<0.05，thus use 0.05 instead of 0.03 in next calculation)

Cut-off Value: 0.05 + 0.1 = 0.15

CONTROL PROCEDURE

The recommended control requirement for this assay involves using positive and negative controls to verify assay performance. The result is valid if the following criteria for the controls are both met:

1. Negative Control

The mean absorbance of negative control is less than 0.1 at 450/630 nm or at 450 nm after blanking.

2. Positive Control

The mean absorbance of positive control is equal to or greater than 0.7 at 450/630 nm or at 450 nm after blanking.

INTERPRETATION OF RESULTS

1. Nonreactive Results

Samples giving an absorbance less than the cut-off value are considered nonreactive.

2. Reactive Results

Samples giving an absorbance equal to or greater than the cut-off value are considered initially reactive by the criteria of the Anti-HIV CLIA assay.

All samples that are reactive on initial testing must be centrifuged prior to retesting. Initially reactive samples must be retested in duplicate using the Anti-HIV CLIA Assay.

- If the sample result for both retests is less than the Cut-off value, the sample is nonreactive. Nonreactive samples are considered negative for Anti-HIV by the criteria of the Anti-HIV CLIA.
- If the sample result for either duplicate retest is greater than or equal to the Cut-off value, the sample is considered repeatedly reactive. Repeatedly reactive results indicate the presence of Anti-HIV by the criteria of the Anti-HIV CLIA.

PERFORMANCE CHARACTERISTICS

1. MEASUREMENT PRECISION

This assay is designed to have a within-run precision of <10%. 1 human serum based panel member was assayed, using 1 batch of reagents, in replicates of 10, once daily for three days. Data from this study are summarized in the following table.

Panel Member	Batch	n	Mean	Within-run Precision	
				SD	%CV
1	1	30	1.120	0.089	7.99
1	1	30	1.072	0.077	7.17
1	1	30	1.187	0.081	6.82

This assay is designed to have a within-run precision of <15%. 1 human serum based panel member was assayed, using 3 batches of reagents, in replicates of 10. Data from this study are summarized in the following table.

Panel Member	Batch	n	Mean	Within-run Precision	
				SD	%CV
1	1	30	1.350	0.081	6.01

2. SENSITIVITY

The diagnostic sensitivity of the product was determined by testing a panel of 600 positive samples, all samples resulted positive; therefore the diagnostic sensitivity was 100%.

3. SPECIFICITY

A total of 3117 fresh serum and plasma samples from volunteer blood donors and medical examination donors were collected and tested at different geographically distinct blood centers and hospitals. Of the 3117 samples, 6 (3111/3117) samples were repeatedly reactive, and based on supplemental test results from a licensed and/ or research immunoblot assay, they were Anti-HIV negative. Thus this assay has a 99.81% specificity.

LIMITATIONS OF THE PROCEDURE

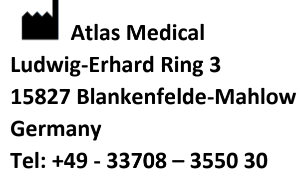
1. This assay is intended as an aid for the clinical diagnosis. The clinical examination, the patient's medical history and other test results must also be taken into account when diagnosing HIV infection.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. False-reactive test results can be expected with any test kit. False-reactive test results have been observed due to nonspecific interactions.
4. Some samples that have undergone multiple freeze-thaw cycles or have been stored frozen for prolonged periods may result in erroneous or inconsistent test results
5. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required

for diagnosis. This kind of samples is not suitable to be tested by this assay.

6. Performance of this test has not been established with neonatal samples.
7. This assay was designed and validated for use with human serum and plasma from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.

REFERENCES

1. Barré-Sinoussi F, Chermann JC, Rey F, et al. (1983).Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science;220:868-871.
2. HIV and its transmission. Centers for Disease Control and Prevention HIV/AIDS Fact Sheets Web site. http://www.cdc.gov/hiv/resources/fac-tsheets/transmission.htm. Published July 1999. Accessed November 21,2009.
3. Leligdowicz A, Yindom L-M, Onyango C, et al. (2007) Robust Gag-specific T cell responses characterize viremia control in HIV-2 infection. J. Clin. Invest. 117(10):3067-3074.
4. Cohen, MS; Hellmann, N; Levy, JA; DeCock, K; Lange, J (2008). "The spread, treatment, and prevention of HIV-1: evolution of a global pandemic". The Journal of Clinical Investigation118 (4): 1244–54.
5. Tao G, Branson BM, Kassler WJ, Cohen RA. (1999) Rates of Receiving HIV Test Results: Data From the U.S. National Health Interview Survey for 1994 and 1995. JAIDS Journal of Acquired Immune Deficiency Syndromes. 22(4).
6. Kelly JA, Otto-Salaj LL, Sikkema KJ, Pinkerton SD, Bloom FR. (1998) Implications of HIV treatment advances for behavioral research on AIDS: Protease inhibitors and new challenges in HIV secondary prevention. Health Psychology.17 (4):310-319.



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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry