HIV 1+2 Ab ELISA Kit

In vitro Diagnostics

CAT NO: S024-04

INTENDED USE

The Sensit HIV 1+2 Ab ELISA Kit is a solid phase enzyme linked-immunosorbent assay for the qualitative detection of anti-HIV-1 including subtype O and anti-HIV-2 antibodies (including isotype IgG, IgM and IgA) in human serum or plasma. It is intended for professional use only as an aid in the diagnosis of infection with HIV-1 and HIV-2 viruses. Any reactive specimen with the Sensit HIV 1+2 Ab ELISA Kit must be confirmed with alternative testing method(s) and clinical findings.

SUMMARY

Human immunodeficiency virus type I and type II (HIV1+2) are enveloped retrovirus containing 2 copies of single stranded RNA genome. Most people diagnosed with HIV will develop AIDS within 10 years if left untreated. HIV-1 has been isolated from patients with AIDS and AIDS related complex, and from healthy individuals with a high risk for developing AIDS. HIV-2 has been isolated from West African AIDS patients and from seropositive asymptomatic individuals. Infection with HIV induces the immune system to produce antibodies against viral proteins of HIV genome, ENV, GAG and POL. Diagnosis of anti-HIV seropositivity is based on the detection of these specific antibodies. The Sensit HIV 1+2 Ab ELISA Kit is for the qualitative detection of the presence of anti-HIV-1 and HIV-2 antibodies.

TEST PRINCIPLE

Sensit HIV 1+2 Ab ELISA Kit is a solid phase enzyme linked immunosorbent assay based on the principle of the double antigen-sandwich technique for the detection of the various antibodies against HIV-1 and/or HIV-2 in human serum or plasma.

The Sensit HIV 1+2 Ab ELISA Kit is composed of two key components:

1) Solidmicrowells pre-coated with recombinant HIV-1 and HIV-2 antigens;
2) Liquid conjugates composed of recombinant HIV-1 and HIV-2 antigens conjugated with horse radish peroxidase (HIV 1+2 HRP conjugates).

During the assay, the test specimen is first incubated with the coated microwells. The anti-HIV-1 and anti-HIV-2 antibodies, if present in the specimen, bind to the antigens coated on the microwell surface. In the second incubation with the HRP- HIV 1+2 conjugates, the anti-HIV-1 and anti-HIV-1 antibodies absorbed on the surface of microwell react to the HRP-HIV 1+2 conjugates. Unbounded conjugates are then removed by washing. The presence of the complexed conjugates is shown by a blue color upon additional incubation with TMB substrate. The reaction is stopped with Stop Solution and absorbance are read using a spectrophotometer at 450 /620-690 $_{nm}$.

REAGENTS & MATERIALS PROVIDED

Item	Description	Quantity	Lot No:
1.	Microwells coated with HIV-1 & HIV-2 antigen	12 strips x 8 wells	MW001-01
2.	HIV Ab Negative Control	1 mL	BS02404-01N
3.	HIV Ab Positive Control	1 mL	BS02404-02P
4.	Sample Diluent	6 mL	ER001-01
5.	HIV 1+2 HRP Conjugates	12 mL	ER002-01
6.	Wash Buffer (30 X)	20 mL	ER004-01
7.	TMB Substrate A	6 mL	ER005-01
8.	TMB Substrate B	6 mL	ER005-02
9.	Stop Solution	12 mL	ER006-01
10.	ELISA Working Sheet	2 Nos	ES001-01
11.	Product Insert	1 No	PIS02401-01

Materials and reagents required but not provided in the kit

1) 50µl and 100µl volume pipette with precision better than 1.5%.
2) Distilled or De-ionized water
3) Microplate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable.
4) Absorbent paper for blotting the microplate wells.
5) Parafilm or other adhesive film for sealing the plate.
6) Timer
7) Incubator

STORAGE & STABILITY

Return all reagents requiring refrigeration immediately after use. All reagents except the concentrated wash buffer are ready to use as supplied. Reseal the microwells after removing the desired number of wells. All the reagents are stable through the expiration date printed on the label if not opened. Store the test kit over 8°C till the expiration date indicated on the pouch / carton. DO NOT FREEZE. Ensure that the test device is brought to room temperature before opening.

PRECAUTIONS & WARNING

1) This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2) Do not use expired devices.
3) Bring all reagents to room temperature (18°C-28°C) before use.
4) Do not use hemolized blood specimen for testing.
5) Do not use the components in any other type of test kit as a substitute for the components in this kit.
6) In the beginning of each incubation and after adding Stopping Solution, gently rocking the microwells to ensure thorough mixing. Avoid the formation of air bubbles as which results in inaccurate absorbance values. Avoid splash liquid while rocking or shaking the wells
7) Don't allow the microplat to dry between the end of the washing operation and the reagent distribution.
8) Do not ingest the reagents. Avoid contact with eyes, skin and mucose. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
9) Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
10) Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
12) Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
13) The enzyme reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or substrate solution.
14) The substrate solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The Substrate B must be stored in the dark.
15) The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.
16) Avoid strong light during color development.
17) Dispose of all specimens and materials used to perform the test as biohazardous waste.

SAMPLE COLLECTION & PREPARATION

1) Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique
2) This kit is designed for use with serum or plasma specimen without additives only.
3) If a specimen is not tested immediately, refrigerated at 2°C-8°C. If storage period greater than three days are anticipated, the specimen should be frozen (-20°C). Avoid repeated freezing-thawing of specimens. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
4) Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.
5) Do not use serum specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

REAGENT PREPARATION

1) Bring all reagents, controls to room temperature (18°C-28°C).
2) Dilute concentrated Wash Buffer 30 X withwater as following:

Plate	DI water	30X wash buffer	Final Volume
Full plate	580 mL	20m L	600 mL
Half plate	290 mL	10 mL	300 mL
A quarter plate	145 mL	5 mL	150 mL

If precipitant appears, warm up the concentrated wash buffer at 37°C.

3) Reagents should be mixed well before adding to the test wells.
4) Mark on the ELISA Working sheet with appropriate information after determining the number of microwells needed. Positive and Negative Controls require to be run in duplicate to ensure accuracy.

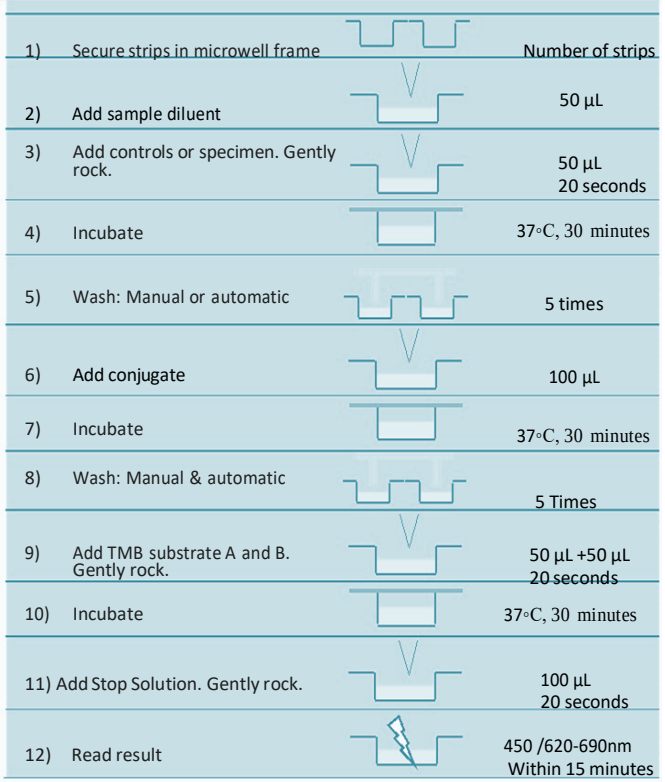
ASSAY PROCEDURE

1) Remove the desired number of strips and secure them in the microwell Frame. Reseal un-used strips.
2) Add specimens according to the designation on the ELISA Working Sheet
   - 2.1 Blank wells: Leave the blank wells alone (2 wells). Don't add any reagents.
   - 2.2 Add 50µL of sample diluent except blank wells
   - 2.3 Control wells: Add 50 µL of HIV Ab Positive Control (2 wells), Negative Control (2 wells) into the designated control wells, respectively.
   - 2.4 Test wells: Add 50 µL of test specimens into each test well, respectively.

   To ensure better precision, use pipette to handle solution.
3) Cover the plate with sealant and incubate the wells at 37◦C for 30 minutes.
4) Carefully remove the incubation mixture by empting the solution into a waste container. Fill each well with diluted wash buffer (350 µL per well) and shake gently for 20-30 second. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 4 more times.
5) Add 100 µL of HRP-antigen conjugate into each well except the blank wells, cover the plate.
6) Incubate the wells at 37 °C for 30 minutes.
7) Wash the plate 5 times as described in step 4.

8) Add 50 µL of TMB substrate A and 50 µL of TMB substrate B into each well including the blank well.
9) Incubate at 37 °C in dark for 30 minutes.
10) Stop the reaction by adding 100 µL of stop solution to each well. Gently mix for 20-30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
11) Set the microplate reader wavelength at 450 nm and measure the absorbance (OD) of each well against the blank well within 15 minutes after adding Stop Solution. A filter of 620–690 nm can be used as a reference wavelength to optimize the assay result.

FLOW CHART OF ASSAY PROCEDURE



INTERPRETATION OFRESULTS

A. Set up the cut-off value

The cutoff value = 0.13 + NC
NC: Mean OD value of Negative Control.

B. Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the Cut-off Value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cut off Value}}$$

C. Assay validation

The mean OD value of the HIV Ab positive controls should be ≥0.80
The mean OD value of the HIV Ab negative controls should be ≤ 0.10.
If above specification are not met, the assay is Invalid. Check the assay procedure including incubation time and temperature and repeat assay.

D. Interpretation of the results

	Specimen OD ratio
Negative	< 1.00
Positive	≥ 1.00

1) The negative result indicates that there is no detectable HIV Ab in the specimen
2) Results just below the cut-off value (Lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to retest in duplicate the corresponding specimens when it is applicable).
3) Specimens with cut-off ≥ 1 are initially considered to be positive by the Sensit HIV 1+2 ELISA kit. They should be retested in duplicate before the final interpretation.
If after re-testing of a specimen, the absorbance value of the 2 duplicates are less than the cut-off value, the initial result is non repeatable and the specimen is considered to be negative with the Sensit HIV 1+2 Ab ELISA Kit. Non repeatable reactions are often caused by:
- Inadequate microwell washing,
- Contamination of the substrate solution by oxidizing agents (bleach, metal ions, etc.)
- Contamination of negative specimens by serum or plasma with a high antibody titer,
- Contamination of the stopping solution

If after re-testing the absorbance of one of the duplicates is equal or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the Sensit HIV 1+2 AB ELISA Kit, subject to the limitation of the procedure, described below.

PERFORMANCE CHARACTERISTICS

Clinical Performance

A total of 1095 patient specimens from suspectible subjects were tested by the Sensit HIV 1+2 Ab ELISA kit. Comparison for all the subjects is showed in the following table:

Ref HIV 1+2 Ab ELISA	Positive	Negative	Total
Positive	58	0	58
Negative	1	1036	1037
Total	59	1036	1095

Relative Sensitivity: 100%, Relative Specificity :99.9%, Overall Agreement: 99.9%

LIMITATION OF THE TEST

1) The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of anti-HIV antibodies in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2) The Sensit HIV 1+2 Ab ELISA Kit is limited to the qualitative detection of HIV Abs in human serum or plasma. The intensity of color does not have linear correlation with the antigen titer in the specimen.
3) A negative result for an individual subject indicates absence of detectable anti-HIV-1 and HIV-2 antibodies. However, a negative test result does not preclude the possibility of exposure to or infection with HIV-1 and HIV-2.
4) A negative result can occur if the quantity of anti-HIV-1 and HIV-2 Ab present in the specimen is below the detection limits of the or the Abs that are detected are not present during the stage of disease in which a specimen is collected.
5) Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6) The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1) Chang, SY, Bowman, BH, Weiss, JB, Garcia, RE and White, TJ. The origin of HIV-1 isolate HTLV-IIIB. Nature (1993)3/363:466-9
2) Arya, SK, Beaver, B, Jagodzinski, L, Ensoli, B, Kanki, PJ, Albert, J, Fenyo, EM, Biberfeld, G, Zagury, JF and Laure, F. New human and simian HIV-related retroviruses possess functional transactivator (tat) gene. Nature (1987) 328:548-550.

Key to symbols used			
	Manufacturer		Expiration/use by date
	Do not reuse		Date of manufacture
	Consult IFU [Instructions For Use]	LOT	Batch code
	Temperature limitation 2-30°C	IVD	In Vitro diagnostic medical device
	Contains sufficient for 'X ' kits		Do not use if package is damaged
	Keep dry	REF	Catalogue No

Manufactured by,

ubio Biotechnology Systems Pvt Ltd.
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