TRUSTwell®

HIV Ag-Ab ELISA Kit (4th Generation)

REF

AE0420

IVD

- 96-well ELISA kit for the qualitative detection of HIV-1 P24 antigen, anti-HIV-1 Including subtype O and anti-HIV-2 antibodies in human serum or plasma
- Store at 2-8 °C upon receipt

INTENDED USE

The TRUSTwell HIV Ag-Ab ELISA Kit is a solid phase enzyme linked immunosorbent assay for the qualitative detection of HIV-1 P24 antigen, 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 early identification of infection with HIV-1 and HIV-2 viruses. Any reactive specimen with the TRUSTwell HIV Ag-Ab ELISA Kit must be confirmed with alternative testing method(s) and clinical findings

INTRODUCTION

Human immunodeficiency virus type I and type II (HIV1+2) are enveloped single stranded RNA positive virus. The causative relationship between HIV1+2 virus and acquired immunodeficiency syndrome (AIDS) has been established over decades. HIV-1 has been isolated from patients with AIDS and AIDS-related complex, and from healthy individuals with a high risk for developing AIDS (1). HIV-2 has been isolated from West African AIDS patients and from seropositive asymptomatic individuals (2).

Infection with HIV induces the immune system to produce antibodies against viral proteins from different parts of the HIV genome, ENV, GAG and POL. Diagnosis of anti-HIV seropositivity is based on the detection of these specific antibodies. HIV antigen is produced during the viral replication phase and generally appears some days after exposure then decreases quickly as antibodies are being produced. Years later, antigenemia may again increase, and is indicative of intense viral replication. Kits to detect markers of HIV infection have been available since 1985.

The TRUSTwell HIV Ag-Ab ELISA Kit is a latest generation of HIV kits for the simultaneous detection of the presence of anti-HIV antibodies and HIV antigens.

TEST PRINCIPLE

The TRUSTwell HIV Ag-Ab ELISA Kit is a solid phase enzyme linked immunosorbent assay based on the principle of the double antibody/antigen sandwich technique for the detection of HIV-1 P24 antigen and various antibodies against HIV-1 and/or HIV-2 antigen in human serum or plasma.

The TRUSTwell HIV Ag-Ab ELISA Kit is composed by two systems:

1) Solid microwells pre-coated with
   Recombinant HIV-1 and HIV-2 antigens and anti-P24 antibody

2) Liquid conjugates composed of
   a. Recombinant HIV-1 and HIV-2 antigens conjugated with horse radish peroxidase (HRP-HIV 1+2 conjugates) and avidin conjugated with HRP (HRP-avidin conjugates)
   b. Anti-P24 antibody conjugated with biotin (Biotinylated P24 Ab)

During the assay, the test specimen is first incubated with the coated microwells and followed by incubation with biotinylated P24 Ab. The anti-HIV-1 and anti-HIV-2 antibodies, if present in the specimen, bind to the antigens coated on the microwell surface. If P24 present in the specimen, binds to the anti-P24 antibody on the microwell surface and biotinylated P24 Ab in the solution.

In the next incubation with the HRP-HIV 1+2 and HRP-avidin conjugates, the above bounded anti-HIV-1 and anti-HIV-2 antibodies and bounded P24 antigen reactive to the HRP conjugates, forming HRP-complex. 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 450/620-690nm.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	Microwells coated with anti-P24 Ab, recombinant HIV-1 and HIV-2 antigen	12 strips x 8 wells	AE0420W
2	Biotinylated P24 Ab	12 mL	AE0420BP
3	HIV Ab positive control	1 mL	AE0420P1
4	HIV Ag positive control	1 mL	AE0420P2
5	HIV negative control	1 mL	AE0420N
6	HRP–avidin, HIV 1+2 conjugate mixture	12 mL	AE0420H
7	Sample Diluent	6 mL	AE0420SD
8	Wash buffer (30 x concentrate)	20 mL	AWE3000
9	TMB substrate A	6 mL	ATME2000A
10	TMB substrate B	6 mL	ATME2000B
11	Stop solution	12 mL	ASE1000
12	ELISA Working Sheet	2 Nos	AE0001ES
13	Product insert	1 No.	PI-AE0420
14	Microplate sealers	3 No.	
15	Desiccant	4 No.	

Materials and reagents required but not provided in the kit

1. Pipette capable of delivering 25 µL, 50 µL, 75 µL, and 100 µL volumes with a precision better than 1.5%.
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable
3. Absorbent paper for blotting the microplate wells.
4. Parafilm or other adhesive film sealant for sealing plate.
5. Timer.
6. Distilled or de-ionized water.
7. Incubator

STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Return all reagents requiring refrigeration immediately after use. Reseal the microwells after removing the desired number of wells. Ensure that the reagents are brought to room temperature before opening. All the reagents are stable through the expiration date printed on the label if not opened. Do not freeze the kit or expose the kit over 8°C.

WARNING AND PRECAUTIONS

For *in Vitro* Diagnostic Use

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 the components in any other type of test kit as a substitute for the components in this kit.
5. Do not use hemolized blood specimen for testing.
6. 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.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Dispose of all specimens and materials used to perform the test as biohazardous waste.
10. 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
11. Don't allow the microplate to dry between the end of the washing operation and the reagent distribution.
12. 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.
13. 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.
14. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
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.

SPECIMEN COLLECTION AND PREPARATION

- Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
- This kit is designed for use with serum or plasma specimen without additives only.
- 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.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.
- Do not use serum specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PREPARATION OF THE REAGENTS

1. Bring all reagents, controls to room temperature (18°C-28°C).

2. Dilute concentrated Wash Buffer 30 fold with water as following:

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

Warm up the concentrated Wash Buffer at 37°C to dissolve the precipitant if it appears.

3. Mix each reagent before adding to the test wells.

4. Determine the number of microwells needed and mark on the ELISA Working Sheet with the appropriate information. 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 50 µL of sample diluent into all the wells except blank.
2.3 Control wells: Add 100 µL of Negative Control (2 wells), HIV Ab Positive (1 well) and HIV Ag Positive Controls (1 well) into the designated control wells, respectively.
2.4 Test wells: Add 100 µL of test specimen into each test well, respectively.

To ensure better precision, use pipette to handle solution.

3. Mix well and Incubate the plate 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 seconds. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 4 more times.

5. Add 100 µL of the Biotinylated P24 Ab to each above well, but not the Blank well. Gently rock the plate wells for 20 seconds, then cover the plate with sealant and incubate the wells at 37°C for 15 minutes.

6. Discard the solution completely and tapping the plate on fresh absorbent paper (do not wash).

7. Add 100 µL of HRP-conjugate Mixture into each well, but not the Blank well, cover the plate, and incubate at 37°C for 30 minutes.

8. Wash the plate 5 times as step 4 described.

9. Add 50 µL of TMB substrate A and 50 µL of TMB substrate B into each well including the Blank well.

10. Incubate at 37°C in dark for 15 minutes.

11. Stop the reaction by adding 100 µL of stop solution to each well. Gently mix for 20 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**

12. 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		
1.	Secure strips in microwell frame	Number of strips
2.	Add sample diluent	50 µL
3.	Add controls or specimens Gently rock	100 µL 20 seconds
4.	Incubate	37°C, 30 minutes
5.	Wash: manual or automatic	5 times
6.	Add Biotinylated P24 Ab Gently rock	100 µL 20 seconds
7.	Incubate	37°C, 15 minutes
8.	Discard the solution Do not wash	
9.	Add Conjugate Gently rock	100 µL 20 seconds
10.	Incubate	37°C, 30 minutes
11.	Wash: manual or automatic	5 times
12.	Add TMB substrate A and B Gently rock	50 µL + 50 µL 20 seconds
13.	Incubate	37°C, 15 minutes
14.	Add Stop Solution. Gently rock	100 µL, 20 seconds
15.	Read result	450/620-690 nm within 15 minutes

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cutoff value = **0.15 + NC**

NC: Mean OD of the negative control. Use 0.05 for calculation of the cut-off value if less than 0.05.

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:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cutoff Value}}$$

C. Assay validation

The mean OD value of the HIV negative controls ≤ 0.10.
The mean OD value of the HIV Ab positive controls ≥ 0.80.
The mean OD value of the HIV Ag positive controls ≥ 0.80.
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 antibody and antigen 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 ≥ 1.00 are initially considered to be positive by the TRUSTwell HIV Ag-Ab ELISA Kit. They should be retested in duplicate before 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 TRUSTwell HIV Ag-Ab ELISA Kit.
   Non repeatable reactions are often caused by:
   - Inadequate microwell washing,
   - Contamination of negative specimens by serum or plasma with a high antibody titer,
   - Contamination of the substrate solution by oxidizing agents (bleach, metal ions, etc.)
   - Contamination of the stopping solution

   If after retesting 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 TRUSTwell HIV Ag-Ab ELISA Kit, subject to the limitation of the procedure, described below.

PERFORMANCE CHARACTERISTICS

1. Clinical Performance

A total of 1100 patient specimens from susceptible subjects were tested by the TRUSTwell HIV Ag-Ab ELISA Kit. Comparison for all subjects is showed in the following table:

	TRUSTwell HIV Ag-Ab ELISA Kit		
Ref. EIA	**Positive**	**Negative**	**Total**
Positive	83	0	83
Negative	1	1016	1017
Total	84	1016	1100

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

2. HIV-1 p24 Analytical Sensitivity

During the internal evaluation of three different batches, the HIV-1 p24 antigen limit of detection was established at 5 IU/mL by testing the WHO HIV-1 p24 Antigen 1st International Standard, NIBSC Code No. 90/636.

LIMITATION OF THE TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of HIV antigen and anti-HIV antibodies in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The TRUSTwell HIV Ag-Ab ELISA Kit is limited to the qualitative detection of HIV antibody and antigen in human serum or plasma. The intensity of the color does not have linear correlation with antibody and antigen titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable HIV P24 antigen, 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 P24 antigen, anti-HIV-1 and HIV-2 antibodies present in the specimen is below the detection limits of the assay, or the antigen and antibodies 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
3. Caetano JA Immunologic aspects of HIV infection. Acta Med Port (1991) 4 Suppl 1:52S-58S
4. Janssen, RS, Satten, GA, Stramer, SL, te al. New testing strategy to detect early HIV-1 infection for use in incidence estimates and for clinical and prevention purposes. JAMA (1998) 280(1): 42-48