## HIV 1+2 Ab ELISA Kit

REF

AE0410

- 96-well ELISA kit for the qualitative determination of 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 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 TRUSTwell HIV 1+2 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.
The TRUSTwell HIV 1+2 Ab ELISA Kit is a third generation of HIV kits for the qualitative detection of the presence of anti-HIV-1 and HIV-2 antibodies.

## TEST PRINCIPLE

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

1) Solid microwells 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.

## MATERIALS AND REAGENTS

**Materials and reagents provided with the kit**

| Item | Description | Quantity | Catalog |
|---|---|---|---|
| 1 | Microwells coated with HIV-1 & HIV-2 antigen | 8 wells x 12 strips | AE0410W |
| 2 | HIV Ab positive control | 1 mL | AE0410P |
| 3 | HIV Ab negative control | 1 mL | AE0410N |
| 4 | Sample Diluent | 6 mL | AE0410SD |
| 5 | HIV 1+2 HRP conjugates | 12 mL | AE0410H |
| 6 | Wash buffer (30 x concentrate) | 20 mL | AWE3000 |
| 7 | TMB substrate A | 6 mL | ATME2000A |
| 8 | TMB substrate B | 6 mL | ATME2000B |
| 9 | Stop solution | 12 mL | ASE1000 |
| 10 | ELISA working sheet | 2 Nos | AE0001ES |
| 11 | Product insert | 1 No. | PI-AE0410 |

**Materials and reagents required but not provided in the kit**

1. Pipette capable of delivering 50 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 450 nm 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. Refrigerate all the reagents 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 splashing 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

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.

## 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: Don't add any reagents into the Blank wells (2 wells).
   - 2.2 Add 50 µL 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 specimen into each test well, respectively.

   *To ensure better precision, use pipette to handle solution.*
3. Cover the plate with sealant. Incubate the wells at 37°C for 30 minutes.
4. Carefully remove the incubation mixture by emptying 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 at 37°C for 30 minutes.

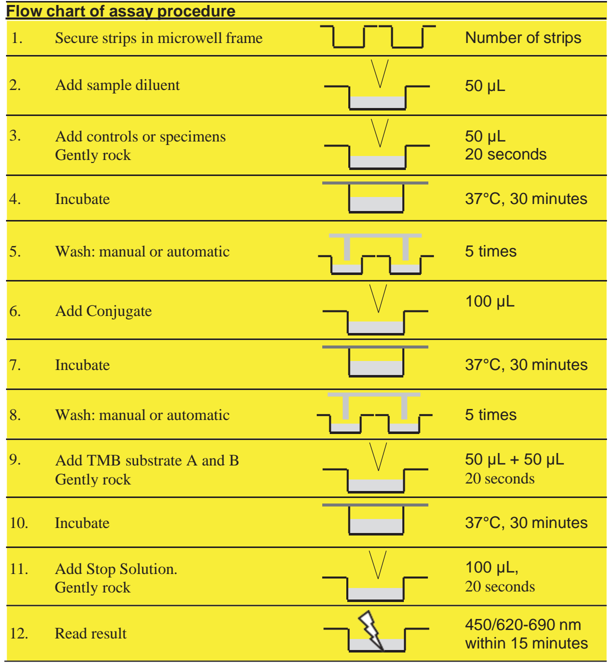
7. Wash the plate 5 times as step 4 described.

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 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**

| Step | | Amount / Conditions |
|---|---|---|
| 1. | Secure strips in microwell frame | Number of strips |
| 2. | Add sample diluent | 50 µL |
| 3. | Add controls or specimens<br>Gently rock | 50 µL<br>20 seconds |
| 4. | Incubate | 37°C, 30 minutes |
| 5. | Wash: manual or automatic | 5 times |
| 6. | Add Conjugate | 100 µL |
| 7. | Incubate | 37°C, 30 minutes |
| 8. | Wash: manual or automatic | 5 times |
| 9. | Add TMB substrate A and B<br>Gently rock | 50 µL + 50 µL<br>20 seconds |
| 10. | Incubate | 37°C, 30 minutes |
| 11. | Add Stop Solution.<br>Gently rock | 100 µL,<br>20 seconds |
| 12. | Read result | 450/620-690 nm<br>within 15 minutes |

## INTERPRETATION OF RESULTS

**A. Set up the cut-off value**

The cut-off value = **0.13 + NC**
NC: Mean OD of the 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 antibody 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 1+2 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 1+2 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 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 susceptible subjects were tested by the TRUSTwell HIV 1+2 Ab ELISA Kit. Comparison for all subjects is showed in the following table:

| | TRUSTwell HIV 1+2 Ab ELISA Kit | | |
|---|---|---|---|
| **Ref. EIA** | **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 TRUSTwell HIV 1+2 Ab ELISA Kit is limited to the qualitative detection of HIV antibodies in human serum or plasma. The intensity of color does not have linear correlation with the antibody 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 antibodies present in the specimen is below the detection limits of the assay, or the 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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