

For Research Use Only Not for Diagnostic Use



HIV 1 + 2
Catalog #: WI4396

HUMAN IMMUNODEFICIENCY VIRUSES ANTIBODY
ELISA KIT
Two-Step Incubation, Double Antigen Sandwich
Principle

INSTRUCTIONS FOR USE

This HIV 1+2 ELISA is an enzyme-linked immunosorbent assay (ELISA) for qualitative determination of antibodies to Human Immunodeficiency Virus (HIV) type 1 and/or type 2 in human serum or plasma. For Research Use Only

SUMMARY

The human immunodeficiency viruses type 1 and type 2 are the etiological agents of the acquired immunodeficiency syndrome (AIDS) and related conditions. HIV has been isolated from patients with AIDS, AIDS related complex (ARC) and from healthy individuals at high risk for AIDS. Infection with HIV is followed by an acute flu-like illness. This phase may remain unnoticed and the relationship to HIV infection may not be clear in many cases. The acute phase is typically followed by an asymptomatic carrier state, which progresses to clinical AIDS in about 50% of infected individuals within 10 years after seroconversion.

Serological evidence of infection with HIV may be obtained by testing for presence of HIV antigens or antibodies in serum of individuals suspected for HIV infection. Antigens can generally be detected during both acute phase and the symptomatic phase of AIDS only. The Antibodies to HIV-1 and/or HIV-2 can be detected throughout virtually the whole infection period, starting at, or shortly after the acute phase and lasting till the end stage of AIDS. Therefore, the use of highly sensitive antibody assays is the primary approach in serodiagnosis of HIV infection. Apart from sexual transmission, the principal route of infection with HIV is blood transfusion. HIV can present both in cellular and cell-free fractions of human blood.

PRINCIPLE OF THE ASSAY

This HIV 1+2 ELISA is a two step incubation antigen "sandwich" enzyme immunoassay kit, which uses polystyrene microwell strips pre-coated with recombinant HIV antigens expressed in E.coli (recombinant HIV-1gp41, gp120, and recombinant HIV-2 gp-36). Patient's serum or plasma sample is added, and during the first incubation

step, the specific HIV1/2 antibodies will be captured inside the wells if present. The microwells are then washed to remove unbound serum proteins. A second recombinant antigen conjugated to Horseradish Peroxidase (HRP-Conjugate) and expressing the same epitopes as the pre-coated antigen is added, and during the second incubation, this antigen will bind to the captured antibody. The microwells are washed to remove unbound conjugate, and Chromogen solutions are added to the wells. In wells containing the antigen-antibody-antigen (HRP) "sandwich" immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HIV 1/2 remain colorless.

COMPONENTS

96 Tests

- **MICROWELL PLATE** 1 plate
Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. **8×12/12×8-well** strips wells per plate. Each well contains recombinant HIV 1/2 antigens. The microwell strips can be broken to be used separately. Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return to 2~8°C.
- **NEGATIVE CONTROL** 1 vial
Yellowish liquid filled in a vial with green screw cap. 1ml per vial. Protein-stabilized buffer tested non-reactive for HIV 1/2. Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8°C.
- **POSITIVE CONTROL SERUM-1 (HIV 1)** 1 vial
Red-colored liquid filled in a vial with red screw cap 1ml per vial. Antibodies to HIV 1 diluted in protein-stabilized buffer. Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8°C
- **POSITIVE CONTROL SERUM-2 (HIV 2)** 1 vial
Red-colored liquid filled in a vial with yellow screw cap. 1ml per vial. Antibodies to HIV 2 diluted in protein-stabilized buffer Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8°C.
- **HRP-CONJUGATE REAGENT** 1 vial
Red-colored liquid filled in a white vial with red screw cap. 13ml per vial, Horseradish peroxidase-conjugated HIV 1/2 antigens. Ready to use as supplied. Once open, stable for one month at 2-8°C.
- **WASH BUFFER** 1 bottle
Colorless liquid filled in a clear bottle with white screw cap. 50ml per bottle. pH 7.4 20 × PBS. (Containing Tween-20 as a detergent). **DILUTE BEFORE USE** -The concentrate must be diluted **1 to 20** with distilled/deionized water before use. Once diluted, stable for one week at room temperature or for two weeks at 2-8°C.
- **CHROMOGEN SOLUTION A** 1 vial
Colorless liquid filled in a white vial with green screw cap.

8ml per vial. Urea peroxide solution. Ready to use as supplied. Once open, stable for one month at 2-8°C.

● **CHROMOGEN SOLUTION B** 1 vial
Colorless liquid filled in a black vial with black screw cap. 8ml per vial, TMB solution (Tetramethyl benzidine dissolved in citric acid). Ready to use as supplied. Once open, stable for one month at 2-8°C.

● **STOP SOLUTION** 1 vial
Colorless liquid filled in a white vial with yellow screw cap. 8ml per vial, Diluted sulfuric acid solution (2.0M H₂SO₄).

● **PLASTIC SEALABLE BAG** 1 unit
For enclosing the strips not in use.

● **CARDBOARD PLATE COVER** 3 sheets
To cover the plates during incubation and prevent evaporation or contamination of the wells.

● **PACKAGE INSERTS** 1 copy

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED BUT NOT PROVIDED

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Disposable V-shaped troughs.
5. Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
6. Absorbent tissue or clean towel.
7. Dry incubator or water bath, 37±0.5°C.
8. Microshaker for dissolving and mixing conjugate with samples.
9. Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
10. Microwell aspiration/wash system.

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

1. **Sample Collection:** Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22µ filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.
2. **Transportation and Storage:** Store samples at 2-8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for

transport of clinical samples and ethological agents.

SPECIAL INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles of 350-400µl/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. It is recommended that the washing system should be calibrated on the kit itself in order to match the declared analytical performances. Assure that the microplate washer's liquid dispensing channels are not blocked or contaminated, and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to carry out 5 cycles, dispensing 350-400µl/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before liquids are wasted in an appropriate way.

The concentrated Washing solution should be diluted **1 to 20** before use. For one plate, mix 50ml of the concentrate with 950ml of water for a final volume of 1000ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, **do not freeze**. To assure maximum performance of this HIV 1+2 ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

This kit is intended **FOR RESEARCH USE ONLY**

The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on

reagents labels or on the kit box.

3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature (18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth. The use of automatic pipettes is recommended.
10. Assure that the incubation temperature is 37°C inside the incubator.
11. When adding samples, avoid touching the well's bottom with the pipette tip.
12. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
13. All specimens from human origin should be considered as potentially infectious.
14. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV 1/2, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
15. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
16. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps for disposal.
17. The Stop solution (2M H₂SO₄) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
18. The enzymatic activity of the HRP-conjugate might be

affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of such substances.

19. Materials Safety Data Sheet (MSDS) available upon request.
20. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

ASSAY PROCEDURE

- Step 1 Reagents preparation:** Allow the reagents and samples to reach room temperature (**18-30°C**) for at least 15-30 minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed, resolubilize by warming at 37°C until crystals dissolve. Dilute the Wash Buffer **1 to 20** with distilled or deionized water. Use only clean vessels to dilute the buffer.
- Step 2 Numbering Wells:** Set the strips needed in strip-holder and number sufficient number of wells including three for the Negative controls (**e.g. B1, C1, D1**), two for the Positive controls (one for HIV1 and one HIV2 controls- **e.g. E1, F1**) and one Blank (**e.g. A1**, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required.
- Step 3 Adding Samples:** Add **100µl** of Positive controls, Negative controls, and Specimen into their respective wells. (**Note: to avoid cross-contamination use a separate disposable pipette tip for each specimen, Negative or Positive Control**).
- Step 4 Incubating (1):** Cover the plate with the plate cover and incubate for **30minutes at 37°C**. It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.
- Step 5 Washing(1):** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash buffer. Each time, allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the plate down onto blotting paper or clean towel, and tap it as to remove any remaining liquids.
- Step 6 Adding HRP-Conjugate:** Add **100µl** HRP-Conjugate into each well except in the Blank.
- Step 7 Incubating (2):** Cover the plate with the plate cover and incubate for **30 minutes at 37°C**.
- Step 8 Washing (2):** After the end of the incubation, remove and discard the plate cover. Wash each well **5times** with diluted Wash buffer as in **Step5**.
- Step 9 Coloring:** Dispense **50µl** of Chromogen A and **50µl** Chromogen B solution into each well including the **Blank**, cover the plate with plate cover and mix by tapping the plate gently.

Incubate the plate at **37°C for 15 minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in positive control and HIV 1/2 positive sample wells.

Step 10 Stopping Reaction: Remove and discard the plate cover. Using a multichannel pipette or manually, add **50µl** Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and HIV 1/2 positive sample wells.

Step 11 Measuring the Absorbance: Calibrate the plate reader with the Blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**. Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within **15minutes** after stopping the reaction)

INTERPRETATION OF RESULTS AND QUALITY CONTROL

Each microplate should be considered separately when calculating and interpreting the results, regardless of the number of plates concurrently processed. The results are calculated by relating each sample's optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

1. Calculation of the Cut-off value: $C.O. = *NC + 0.12$

*NC = the mean absorbance value for three negative controls

Example:

1. Calculation of NC:

Well No: B1 C1 D1
Negative Controls OD value: 0.032 0.031 0.027
NC=0.030

2. Calculation of Cut-off: $(C.O.) = 0.030 + 0.12 = 0.150$

If one of the Negative Control values does not meet the Quality control range specifications, it should be discarded and the mean value calculating again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

2. Quality control range:

The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
2. The OD value of the Positive control must be equal to

or greater than 0.800 at 450/630nm or at 450nm after blanking.

3. The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.

3. Interpretations of the results:

(S = the individual absorbance (OD) of each specimen)

Negative Results (S/C.O. <1): Samples giving absorbance less than the Cut-off value are negative for this assay, which indicates that no HIV 1/2 antibodies have been detected with this HIV 1/2 ELISA kit

Positive Results (S/C.O. ≥ 1): Samples giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that HIV 1/2 antibodies have probably been detected using this HIV 1/2 ELISA kit. Retesting in duplicates of any initially reactive sample is recommended. **Borderline (S/C.O. =0.9-1.1) :** Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these samples in duplicates is recommended to confirm the results.

LIMITATIONS

1. Non-repeatable positive result may occur due to the general biological characteristics of the ELISA method. The assay is designed to achieve very high performance characteristics of sensitivity and specificity and the "sandwich" model minimizes the unspecific reactions due to interference with unknown matters in sample. Antibodies may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
- 2.
3. Common sources for mistakes are: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
4. The prevalence of the marker will affect the assay's predictive values.
5. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step.
6. The assay cannot distinguish between infections with HIV-1 and HIV-2.
7. This is a qualitative assay and the results cannot be used to measure antibodies concentrations.

INDICATIONS OF INSTABILITY OR DETERIORATION OF THE REAGENTS

1. Values of the Positive or Negative controls, which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results

should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones.

2. If after mixing of the Chromogen A and B solutions into the wells, the, the color of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

VALIDITY

Please do not use this kit beyond the expiration indicated on the kit box and reagent labels.

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