For Research Use Only *Not for Diagnostic Use*

HIV (1+2) Ag&Ab

Catalog #: WI4496

HUMAN IMMUNODEFICIENCY VIRUSES ANTIGEN/ ANTIBODY ELISA KIT
Two-Step Incubation, Double Ag&Ab Sandwich Principle

INSTRUCTIONS FOR USE

This Screening HIV (1+2) Ag&Ab ELISA is an enzyme-linked immunosorbent assay (ELISA) for qualitative determination of antigens or 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.

The 1st generation tests were based on viral lysate antigens derived from viruses that are grown in human T-lymphocyte lines. The presence of traces of host cell components in which the virions have been propagated could lead to cross-contamination and thus to very high rates of false-positive results.

With the cloning of the HIV genome, improved assays based on recombinant proteins and/or synthetic peptides (known as 2nd generation), became rapidly available. The utilization of biotechnology methods allow predominantly expression of the important immunoreactive regions of the proteins and also enabled the production of combined HIV-1/HIV-2 assays. The recombinant antigen could also be produced with considerably more purity and in large amounts, and they can be bond to solid-phase surface with much tighter control over protein ratios and concentrations.

The first and second generations HIV kits were based on indirect ELISA method and could detect IgG antibodies only by enzyme-labeled anti-human IgG antibody.

The third generation ELISA utilized double antigen "sandwich" method: again with antigens coated on solid phase polystyrene plates, but with antibodies detection achieved with the help of another enzyme-labeled antigen. The third generation assays could detect all antibodies in sample (IgG, IgM, etc.) which significantly increases the assay's sensitivity comparing to the previous generations. In addition, the detection of IgM antibodies that are present only during the early stages of infection, much shortens the antibody detection "window" period (the period of time in which there is no detectable antibody production), and compare to the second generation, "sandwich" tests could detect antibodies 11 days earlier.

To reduce even further the antibody detection "window" period, 4th generation HIV ELISAs that could simultaneously detect HIV antigens (p24) and antibodies have been developed and are commercially available since 1998. With detection of p24, the 4th generation tests shorten the "window" period to 16 days, or compare to the 3rd generation, HIV infection could be detected 8 days earlier.

PRINCIPLE OF THE ASSAY

This Screening HIV (1+2) Ag&Ab ELISA is a two-step incubation, "sandwich" enzyme immunoassay kit, which uses polystyrene microwell strips pre-coated with recombinant HIV antigens (recombinant HIV-1 gp41, gp120, and recombinant HIV-2 gp36) and anti-HIV (p24) antibodies.

As a first step, biotinylated anti-HIV (p24) antibodies together with the patient's serum or plasma sample are added into the wells. During incubation, the specific HIV-1/2 antibodies if present in sample, will be captured inside the wells.

Simultaneously, if HIV p24 antigen is present in sample, it will also be captured as a double antibody "sandwich" complex comprising of the coated antibodies-p24-biotinylated antibodies. The microwells are then washed to remove unbound serum proteins.

The detection of the captured HIV p24 antigen-biotinylated antibody complex or HIV-1/2 antibodies is achieved during the second incubation step by adding of the enzyme Horseradish Peroxidase (HRP) which has been conjugated to second HIV 1+2 recombinant antigens and to avidin.

p24 detection:

When p24 has been captured inside the wells, avidin will react with the biotin and attach HRP to the Ab-p24-Ab complex.

HIV-1/2 antibody detection:

When HIV-1/2 antibodies have been captured inside the wells, the HRP-conjugated antigens will bind to the

captured antibodies forming Ag-Ab-Ag (HRP) "sandwich" immunocomplex.

The microwells are washed to remove unbound conjugate, and Chromogen solutions are added to the wells.

In wells containing the Ag-Ab-Ag (HRP) and/or Ab-p24-Ab (HRP) "sandwich" immunocomplexes, the colorless Chromogens are hydrolyzed by the bound HRP 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 antibodies or p24 captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HIV-1/2 or p24 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 and anti-p24 antibodies. 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 2-8ºC.

- **NEGATIVE CONTROL** 1 vial

Yellowish liquid filled in a vial with green screw cap
1 ml 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 2-8ºC.

- **ANTIBODY POSITIVE CONTROL-1 (HIV-1)** 1 vial

Red-colored liquid filled in a vial with red screw cap
1 ml 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 2-8ºC

- **ANTIBODY POSITIVE CONTROL-2 (HIV-2)** 1 vial

Red-colored liquid filled in a vial with yellow screw cap.
1 ml 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 2-8ºC.

- **ANTIGEN POSITIVE CONTROL** 1 vial

Red-colored liquid filled in a vial with blue screw cap.
1 ml per vial. HIV p24 recombinant antigen diluted in protein-stabilized buffer. Preservatives: 0.1% ProClin 300. Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **HRP-CONJUGATE REAGENTS** 1 vial

Red-colored liquid filled in a white vial with red screw cap.
12 ml per vial
- Horseradish peroxidase conjugated HIV-1/2 antigens.
- Horseradish peroxidase conjugated avidin.

Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **BIOTIN-CONJUGATE REAGENT** 1 vial

Blue-colored liquid filled in a vial with blue screw cap.
3 ml per vial. Biotinylated anti-HIV p24 antibodies diluted in protein-stabilized buffer. Preservatives: 0.1% ProClin 300.
Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **STOCK WASH BUFFER** 1 bottle

Colorless liquid filled in a clear bottle with white screw cap.
50 ml 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 2-8ºC.

- **CHROMOGEN SOLUTION A** 1 vial

Colorless liquid filled in a white vial with green screw cap.
6 ml per vial. Urea peroxide solution. Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **CHROMOGEN SOLUTION B** 1 vial

Colorless liquid filled in a black vial with black screw cap.
6 ml per vial, TMB solution (Tetramethyl benzidine dissolved in citric acid). Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **STOP SOLUTION** 1 vial

Colorless liquid filled in a white vial with yellow screw cap.
6 ml per vial, Diluted sulfuric acid solution (2.0M H_2SO_4).

- **PLASTIC SEALABLE BAG** 1 unit

For enclosing the strips not in use.

- **CARDBOARD PLATE COVER** 2 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±1ºC.
8. Microshaker for dissolving and mixing conjugate with samples.
9. Microwell plate reader, single wavelength 450 nm or dual wavelength 450 nm and 630 nm.
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 0.22 μm 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 2-8ºC. Samples not required for assaying within 3 days should be stored frozen (-20ºC 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 350-400 µl/well are sufficient to avoid false positive reactions and high background.
3. To avoid cross-contaminations of the plate with sample or HRP-conjugates, after incubation do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer 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 350-400 µl/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 50 ml of the concentrate with 950 ml of water for a final volume of 1000 ml diluted Wash Buffer. If less than a whole plate

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8ºC, **do not freeze**. To assure maximum performance of this Screening HIV (1+2) Ag&Ab 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 (18-30ºC) before use. Shake reagent gently before, and return to 2-8ºC 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 37ºC 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 450 nm or at 450 nm with reference at 630 nm.
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 121ºC or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps for disposal.
17. The Stop solution (2M H_2SO_4) 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-conjugates 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 37ºC 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 Negative controls **(e.g. B1, C1, D1)**, three Positive controls **(**one for HIV-1, one HIV-2 and one for HIV-Ag controls- **e.g. E1, F1,G1)** and one Blank (**e.g. A1**, neither samples nor HRP-Conjugate should be added into the Blank well). Use only number of strips required.

- **If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted.**
- **Where appropriate, the requirement for use HIV-2 positive control could be omitted.**

Step 3 **Adding Biotin-conjugated reagent:** Add 2**0µl of** biotinylated anti-HIV p24 antibodies into each well except in the Blank.

Step 4 **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 5 **Incubating (1):** Cover the plate with the plate cover and incubate for **60 minutes 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 6 **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 7 **Adding HRP-Conjugate:** Add **100µl** HRP-Conjugates into each well except in the Blank.

Step 8 **Incubating (2):** Cover the plate with the plate cover and incubate for **30 minutes** at **37ºC**.

Step 9 **Washing (2):** After the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash buffer as in **Step 5.**

Step 10 **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 **37ºC for 30 minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP produces blue color in positive control and HIV-1/2 positive for antigens/antibodies sample wells.

Step 11 **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 for antigens/antibodies sample wells.

		This Screening HIV (1+2) Ag&Ab ELISA		
		＋	－	TOTAL
EIA-1 (HIV-Ag/Ab)	＋	297	0	297
	－	0	203	203
	TOTAL	297	203	500
	AGREEMENT ： （297＋203）／500＝100%			
		＋	－	TOTAL
EIA-3(HIV-Ab)	＋	2	1	3
	－	0	2682	2682
	TOTAL	2	2683	2685
	AGREEMENT ： （2＋2682）／2685＝99.96%			
		＋	－	TOTAL
EIA-4(HIV Ag/Ab)	＋	0	1	1
	－	2	2682	2684
	TOTAL	2	2683	2685
	AGREEMENT ： （0＋2682）／2685＝99.89%			

Step 12 **Measuring the Absorbance:** Calibrate the plate reader with the Blank well and read the absorbance at **450 nm**. If a dual filter instrument is used, set the reference wavelength at **630 nm**. Calculate the Cut-off value and evaluate the results. (**Note:** read the absorbance within **10 minutes** 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 450/630 nm or at 450 nm after blanking.
3. The OD value of the Negative control must be less than 0.100 at 450/630 nm or at 450 nm 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 or p24 antigen have been detected with this Screening HIV (1+2) Ag&Ab 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 and/or p24 antigen have probably been detected using this Screening HIV (1+2) Ag&Ab ELISA kit. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive

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 or p24 may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. 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.
3. The prevalence of the marker will affect the assay's predictive values.
4. 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.
5. The assay cannot distinguish between infections with HIV-1 and HIV-2.
6. **T**he assay cannot distinguish between positive antibody and positive p24 antigen results.
7. This is a qualitative assay and the results cannot be use 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 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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