# ANTI HIV Ag/Ab 4th Gen ELISA

| CAT NO | DESCRIPTION | PACK SIZE |
|---|---|---|
| EIAHV41 | Anti HIV Ag/Ab 4th Gen ELISA | 96 Tests |

**Intended Use:**

Anti HIV Ag/Ab ELISA is an Enzyme linked immunosorbent assay intended for the qualitative detection of antigens and/or antibodies to HIV type (group M-O) and/or type 2 in human serum or plasma samples. The method is known in the market as the 4th Generation ELISA assay for the detection of HIV. The kit can be used for the screening of blood donors and as an aid in the diagnosis of clinical conditions related to infection with HIV-1 and HIV-2 the etiological agents of AIDS. This reagent is for In vitro Diagnostic use by trained professionals only.

**Summary and Principle:**

The human immunodeficiency viruses type 1 and type 2 are the etiological agents of 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 the acute phase and the symptomatic phase of AIDS only. Antibodies to HIV-1 and/or HIV-2 can be detected generally throughout the infection period, starting at, or shortly after the acute phase and lasting till the end stage of AIDS. Apart from sexual transmission, the principal route of infection with HIV is via blood transfusion. HIV can present both in cellular and cell-free fractions of human blood. Therefore, all donations of blood or plasma should be tested due to the risk of HIV transmission through contaminated blood.

ELISA tests for detection of HIV infection are characterized with high sensitivity, specificity and simple operational procedure. This assay format is most appropriate for testing of large numbers of specimens and a number of HIV tests used in routine blood screening or clinical diagnosis are commercially available. Since the first HIV ELISA tests were commercially introduced in 1985, four more generations have been developed. The 1st generation tests were based on viral lysate antigens derived from viruses that were 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 available. Using new biotechnology methods enabled expression of the most important immunoreactive regions of the proteins and also allowed for the production of combined HIV-1/HIV-2 assays. The recombinant antigen can now also be produced with considerably more purity and in large amounts, and they can be bound to the 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 i.e. with antigens coated on solid phase polystyrene plates, and another enzyme-labeled antigen in the conjugate. The third generation assays could detect all antibodies in sample (IgG, IgM, etc.) which significantly increases the assay's sensitivity compared to the previous assay incarnations. In addition, the detection of IgM antibodies that are present only during the early stages of infection, significantly improves the antibody detection window period, typically detecting antibodies 11 days earlier compared to the second generation, sandwich tests. To reduce even further the HIV detection window, 4th generation HIV ELISAs have been developed that can simultaneously detect HIV antigens (p24) and antibodies. With detection of p24, the 4th generation tests shorten the detection window to 16 days, with HIV infection detected 8 days earlier. 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. In the first step, biotinylated anti-HIV (p24) antibody together with unknown serum or plasma samples are added to the wells. During incubation, any HIV-1/2 antibodies present in samples, will be captured on the well surface. Simultaneously, if HIV p24 antigen is present in the sample, it will also be bound as a double antibody sandwich complex between the coated and biotinylated anti-HIV p24 antibodies. The plate is washed to remove unbound serum proteins and conjugate. The detection of the captured p24 antigen-biotinylated antibody complex and/or HIV-1/2 antibodies is achieved in the second incubation step by adding conjugate containing Horseradish Peroxidase (HRP) which has been conjugated to HIV 1+2 recombinant antigens and also to avidin.

p24 detection: When p24 has been captured in 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 in the wells, the HRP-conjugated antigens will bind to the captured antibodies forming HRP-labelled Ag-Ab-Ag immunocomplexes.

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) immunocomplexes, the colourless chromogens are hydrolyzed by the bound HRP to a blue coloured product. The blue colour turns yellow after stopping the reaction with sulphuric acid. The degree of colour intensity can be measured spectrophotometrically and is proportional to the concentration of antibodies and/or p24 antigen in the sample. Wells containing samples negative for anti-HIV-1/2 or p24 remain colourless.

**Reagent Composition:**

| COMPONENT | SIZE | DESCRIPTION |
|---|---|---|
| Microwell Plate | 1x96 wells (12x8 well plate) | Each microwell is coated with recombinant HIV1/2 antigens and p24 antibodies. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable for 1 month at 2 - 8°C. |
| Negative Control | 1x1ml | Protein stabilized buffer tested non-reactive for HIV 1 / 2. Ready to use. Once open stable for 1 month at 2 - 8°C. |
| Positive Control 1 | 1x1ml | Protein stabilized buffer dilution of HIV 1 antibodies. Red coloured solution. Once open, stable for 1 month at 2 - 8°C. |
| Positive Control 2 | 1x1ml | Protein stabilized buffer dilution of HIV 2 antibodies. Red coloured solution. Once open, stable for 1 month at 2 - 8°C. |
| P24 Antigen Positive | 1x1ml | Protein stabilized buffer dilution of HIV p24 antigen. Red coloured liquid. Once open, stable for 1 month at 2 - 8°C. |
| Conjugate 1 | 1x3.0ml | Red coloured liquid. Biotinylated anti HIV p24 antibodies diluted in a protein stabilized buffer. Ready to use as supplied. Once open stable for 4 weeks at 2 - 8°C. |
| Conjugate 2 | 1x12ml | Red coloured liquid. HRP conjugated recombinant HIV 1+2 antigens and HRP conjugated avidin. Once open, stable for 1 month at 2 - 8°C. |
| Wash Buffer (20X) | 1x40ml | PBS containing detergent. 20X concentrate. Once open, stable for 1 month at 2 - 8°C. The concentrate must be diluted 1/20 with distilled water before use. Once diluted it is stable at room temperature for a week or two weeks at 2 - 8°C. |
| Substrate Solution A | 1x6ml | Urea peroxide solution. Ready to use. Once open, stable for one month at 2 - 8°C. |
| Substrate Solution B | 1x6ml | TMB Solution. Ready to use. Once open, stable for 1 month at 2 - 8°C. |
| Stop Solution | 1x6ml | Diluted Sulphuric acid solution (0.5M) Ready to use. Once open, stable for 1 month at 2 - 8°C. |

Plastic sealable bag, IFU and plate covers.

**Materials required but not provided:**

Distilled/deionized water, disposable gloves, timer, appropriate waste containers for potentially contaminated materials, dispensing pipettes and tips, dry bath incubator or water bath, plate reader, single wavelength 450nm or dual wavelength 450/630nm and automatic plate washer system.

**Sample Collection:**

Collect samples by separation of serum or plasma from red blood cells after standard venipuncture technique. This assay can be performed using either serum or plasma samples. Separate serum and plasma as soon as possible from RBC to avoid haemolysis. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matter in samples should be removed by centrifugation at 3000 rpm for 10 minutes at room temperature or by filtration.

EDTA, sodium citrate or heparin can be used as anticoagulants for plasma samples, but highly lipaemic, icteric or haemolytic samples should not be used as they give false results in the assay. Do not heat inactivate samples which can cause deterioration of the target analyte.

The AMS Anti HIV Ag/Ab ELISA assay is to be used only for testing serum or plasma samples. Do not use for testing cadaver samples, saliva, urine or other body fluids or pooled (mixed) blood.

Transportation and Storage: store samples at 2 - 8°C. HIV analytes will be stable in samples for up to 7 days at 2 - 8°C. For longer term storage freeze serum and plasma samples at -20°C or lower. Multiple free thaw cycles should be avoided. For shipment, samples should be packaged and labelled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

**Storage and Stability:**

The contents of the kit will remain stable up to expiry date when stored at 2 - 8°C. Do not freeze. Keep all components tightly capped and without any contamination.

**Precautions and Safety:**

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

1. **Do not mix reagents from different lots or use reagents from other commercially available kits. The components of the kit are precisely matched for optimal performance of the tests.**
2. **Make sure that all reagents are within the expiry indicated on the kit box and of the same lot.**
3. **IMPORTANT: Allow all reagents and samples to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 2 - 8°C immediately after use.**
4. **Do not touch the bottom of the microplate or well strips inside or outside; fingerprints or scratches may interfere with the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.**
5. **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.**
6. **Adhere strictly to the incubation times. When making sample and reagent additions, ensure reagents are added to the wells in the same order and that the pipetting time is the same for each reagent.**
7. **Use different disposal pipette tips for each sample and reagents in order to avoid cross-contamination.**
8. **The enzymatic activity of the HRP-conjugate can be affected by dust and chemicals including sodium hypochlorite, acids, alkalis etc. Keep these substances away to prevent contamination of reagents.**
9. **If using fully automated equipment do not cover the plates with the plate cover for incubations and allow the plate washer carry out all aspirations of wash buffer.**
10. **All samples from human origin should be considered as potentially infectious. Adhere to Good Laboratory Practice regulations to ensure personal safety.**
11. **WARNING: Materials from human origin may have been used in the preparation of the Negative Control of 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 samples or reagents are completely absent. Therefore, handle reagents and samples with extreme caution as if all are potentially infectious. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin and fetal calf sera are derived from animals from BSE/TSE free-geographical areas.**
12. **Never eat, drink or smoke, in the laboratory. Never pipette solutions by mouth.**
13. **Dispose of reagents, well contents waste and samples according to local or national regulations. Pipette tips, vials, strips and sample containers should be collected and autoclaved for not less than 2 hours at 121°C or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps of disposal. Solutions containing sodium hypochlorite should never be autoclaved. MSDS available upon request.**
14. **Some reagents may cause toxicity, irritation, burns or have carcinogenic effects as raw materials. Contact with skin and the mucosa should be avoided but not limited to the following reagents: Stop Solution, Chromogen reagents and the Wash Buffer.**
15. **The Stop Solution contains sulphuric acid. Use it with appropriate care. Wipe up spills, immediately and wash with water if comes into contact with the skin or the eyes.**
16. **Proclin 300 is used as preservative and can cause sensation of the skin. Wipe up spills immediately or wash with water if comes into contact with skin or eyes.**
17. **Values for positive and negative controls which are out of the assigned control range, may indicate possible deterioration of the reagents or operator or equipment errors. In such cases, all sample results from the affected assay run should be considered as invalid and the samples must be retested. In the case of consistently erroneous results and proven deterioration or instability of the reagents, immediately discard the reagents in use and use a new kit. Contact the local AMS representative.**

**Procedure:**

**Reagent preparation:**

Allow the reagents to reach room temperature (18-30°C). Check the wash buffer concentration for the presence of salt crystals. If crystals have formed, re-solubilize by warming at 37°C, until crystals dissolve. Dilute the wash buffer (20X) as indicated in the instructions for washing. Use distilled or deionized water and clean vessels to dilute the buffer. All other reagents are ready to use as supplied.

**STEP 1**

**Preparation:** Assign one well as the Blank Well (e.g. A1 – make sure that no conjugates nor any samples are added to the blank well), assign 2 wells for the Negative Control, 3 wells for the Positive Controls and assign wells for the samples. If the results are read using a plate reader having dual wavelength (450 / 630nm) then the Blank well need not be used. Use just the required number of strips for the test.

**STEP 2**

**Addition of samples:** Add 75 µl of Positive control, Negative Control and samples into their respective wells except the Blank. (Note: Use a new pipette tip for each sample, Negative control, Positive control to avoid cross contamination).

**STEP 3**

**Addition of Conjugate 1:** Add 25 µl of Conjugate 1 to all wells except the Blank well. Mix by tapping the plate gently.

**STEP 4**

**Incubation:** Cover the plate with the plate cover and incubate for 60 minutes at 37°C.

**STEP 5**

**Washing:** At the end of the incubation period, remove the plate cover and discard the contents of the microwells. Wash each well 5 times with diluted Wash Buffer. Leave the microwells to soak for 60 seconds for each wash. After the final wash cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

**STEP 6**

**Addition of Conjugate 2:** Add 100 µl of Conjugate 2 into each well except the Blank well.

**STEP 7**

**Incubation:** Cover the plate with the plate cover and incubate for 30 minutes at 37°C.

**STEP 8**

**Washing:** At the end of the incubation period, remove the plate cover and discard the contents of the microwells. Wash each well 5 times with diluted Wash Buffer. Leave the microwells to soak for 60 seconds for each wash. After the final washing cycle, turn down the plate onto a blotting paper or a clean towel and tap it to remove any residual buffer.

**STEP 9**

**Addition of the Substrate**: Add 50 µl of Substrate Solution A and 50 µl of Substrate Solution B into each well including the blank. Incubate the plate at 37°C for 30 minutes. Ensure this incubation is carried out in darkness.

**STEP 10**

**Stopping the Reaction:** Add 50 µl of Stop solution into each well including the blank and mix gently until all blue coloration has turned to yellow.

**STEP 11**

**Measurement:** 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: Absorbances must be read within 10 minutes of adding the Stop Solution).

**Instructions for Washing:**

The Wash Buffer is to be diluted 1/20 before use. Add the contents of the bottle to 760 ml of distilled water. If less than a whole plate is used, prepare the proportional volume of solution.
To avoid the occurrence of false positive reactions and a high background, an automatic 5 wash cycle is recommended with 350 – 400 µl of diluted Wash Buffer used per well per wash. To avoid cross-contamination of the plate with sample or HRP conjugate, do not discard the content of the wells but allow the plate washer to aspirate contnets automatically.
Ensure 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.
For manual washing, carry out 5 wash cycles, dispensing 350 – 400 µl of diluted Wash Buffer per well and aspirating the liquid by inverting the plate and flicking out the buffer. If poor results are observed with high background, increase the soak time each wash.
Treat the liquid aspirated after the reaction from the wells with 2.5% sodium hypochlorite for 24 hours before disposing off in the appropriate way.

**Calculation of results:**

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample absorbance (Abs) with the cut off value (CO) 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 Abs from the absorbances of the samples and the controls. When results are obtained on a dual filter plate reader, do not subtract the blank well Abs from the sample and control absorbances.

**Calculation:**

Cut off value (CO) = Nc + 0.1

(Nc = the mean absorbance value for the Negative Control)

**Validation:**

Blank well: the absorbance must be <0.080 at 450nm.
Positive Control: the absorbance must be >/= 0.800 at 450/630nm or at 450nm after blanking.
Negative control: the absorbance must be <0.100 at 450/630nm or at 450nm after blanking.
If one of the Negative control absorbances does not match the above criteria, this value should be discarded and a mean value should be calculated using the other value. If both negative control absorbances do not meet the criteria, the assay run is invalid and must be repeated.

**Example:**

| | | | |
|---|---|---|---|
| Blank Value | A1: 0.025 | 450nm (blanking is required only when reading with a single filter) | |
| Negative control | 0.022 | 0.024 | |
| Positive control | 2.109 | 2.114 | 2.106 |

Calculation of Nc: (0.022 + 0.024)/2 = 0.023
Calculation of the cut off: 0.1 + 0.023 = 0.123

**Interpretation of the results:**

**Negative Results:** (Abs/CO <1) Samples with an absorbance results less than that of the Cut off value are negative for this assay. This indicates that the sample is non-reactive for HIV 1 / 2 antibodies and p24 antigen and the subject is probably not infected with HIV 1 / 2 and the blood unit does not contain HIV 1 / 2 and could be transfused in case other infectious diseases markers are also absent.
**Positive Results:** (Abs/CO >/= 1) Samples with an absorbance result equal to or greater than the cut off values are considered initially reactive, indicating that HIV 1 / 2 antibodies have been probably detected. All initially reactive samples should be repeated before final interpretation. Repeatedly reactive samples can be considered positive for HIV 1 / 2 antibodies and/or p24 antigen.
**Borderline:** (Abs/CO = 0.9 – 1.1) Samples with absorbance to cut off ratio between 0.9 to 1.1 are considered borderline and retesting of these samples in duplicates is required to confirm the initial results.
Interpretation of repeat testing:

- After re-testing of initially reactive samples, both wells show negative results (Abs/CO <0.9). These samples should be considered negative and the original result must be classified as false positive. As with many sensitive ELISA Assays, false positive results can occur due to several reasons, most of which are connected with, but not limited to inadequate washing.
- After retesting in duplicate one or both wells show positive results. The final result of this sample should be recorded as repeatedly positive. Repeatedly reactive samples should be considered positive for antibodies to HIV 1 / 2 and the blood unit must be discarded.
- After re-testing in duplicate, samples with values close to the cut-off should be interpreted with caution and considered borderline samples, or uninterpretable for the time of testing.

Follow up, confirmation and supplementary testing of any positive sample with other analytical systems such as WB, PCR is required. Clinical diagnosis should not be established using just the results of this ELISA.

**Performance Characteristics:**

**Specificity**: Overall diagnostics Specificity is 99.90%.
**Sensitivity**: Overall sensitivity: 100%
**Cross Reactivity:** There was no cross reactivity observed when samples positive for HAV HBV, CMV and TP were tested in the HIV Ag/Ab 4th Gen ELISA.
**Prozone:** No prozone or interference was observed when samples containing up to 2000 U/ml Rheumatoid Factor were tested in the HIV Ag/Ab 4th Gen ELISA.

**Limitations:**

- Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
- HIV antigens or antibodies may be undetectable during the early stages of the disease. Negative results only indicate that the sample does not contain detectable levels of HIV 1 / 2 antibodies or antigen but does not rule out the possibility of infection with HIV.
- If, after re-testing of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable and interpreted as negative.
- The most common assay errors include: using kits beyond expiry, bad washing technique, contaminated reagents, improper operation of equipment, sample collection issues.
- This assay cannot be utilized to test pooled serum or plasma. This kit can only be used with individual serum or plasma samples.
- This assay is a qualitative assay and the results cannot be used to measure antibody or antigen concentrations. This assay cannot be used to distinguish between infections with HIV 1 and HIV 2.

**References:**


1. Barre-Sinoussi, F et al., (1984) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquiredimmunodeficiency syndrome (AIDS), Science, 220: 868-871.
2. Barbe, F.et al., (1994) Early detection of antibodies to HIV-1 by a third generation enzyme immunoassay. Ann. Biol. Clin. (Paris), 52: 341-345.
3. Constantine,. N., T. et al., (1993) Serologic test for the retroviruses: approaching a decade of evolution. AIDS, 7: 1-13Gnann JW et al. (1987) Science; 237: 1346-1349.


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| REF | Catalogue number | | Temperature limitation |
| | Consult instructions for use | LOT | Batch code |
| IVD | *In vitro* diagnostic medical device | | Use by Date |
| | Manufacturer | | |


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