Anti-HIV1/2

diasino

Antibodies to human immunodeficiency virus 1/2 (ELISA)

REF: DS177758 Σ 96

Intended use (For professional use only)
The DiaSino Anti-HIV1/2 assay is an enzyme-linked immunosorbent assay (ELISA) for the qualitative determination of human immunodeficiency virus in human serum and plasma.

Summary
The human immunodeficiency virus (HIV), the causative agent of Acquired Immunodeficiency Syndrome (AIDS), belongs to the family of retroviruses. HIV can be transmitted through contaminated blood and blood products, through sexual contact or from a HIV infected mother to her child before, during and after birth. Two types of human deficiency viruses, called HIV-1 and HIV-2, have been identified to date.[1,2] Various subtypes of the known HI-Viruses have been described, each of which has a different geographical distribution. HIV-1 can be divided into 3 distantly related groups: group M (for main), group N (for non-M, non-O) and group O (for outlier).[3,4] Based on their genetic relationship, at least 9 different subtypes (A to D, F to H, J, K) have been identified within HIV-1 group M.[5] Recombinant HIV-1 viruses consisting of sequences of 2 or even more different subtypes exist and are spreading epidemically. Antibodies to HIV proteins, indicating the presence of an HIV infection, can be found in the serum usually 6-12 weeks after infection.[6]

Test principle
Double antigen sandwich assay. Total duration of assay: **100 minutes**
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 set of recombinant antigens conjugated to the enzyme Horseradish Peroxidase (HRP-Conjugate) and expressing the same epitopes as the pre-coated antigens is added, and during the second incubation, they will bind to the captured antibody. The microwells are washed to remove unbound conjugate, and Chromogen solutions are added into 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 the reaction is stopped with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for Anti-HIV1/2 remain colorless.

Reagents
Materials provided
- **HIV1/2 Coated Microplate - symbol** HIV1/2 PLATE 8 x 12 strips. Pre-coated with recombinant HIV 1/2 antigens.
- **HIV1/2 Negative Control - symbol** HIV1/2 NC 1 vial, 1.0 mL.
- **HIV-1 Positive Control - symbol** HIV-1 PC 1 vial, 1.0 mL.
- **HIV-2 Positive Control - symbol** HIV-2 PC 1 vial, 1.0 mL.
- **HIV1/2 Enzyme Conjugate - symbol** HIV1/2 CONJ 1 vial, 11 mL.HRP-Conjugate.
- **Substrate - symbol** SUBSTRATE 1 vial, 11mL, ready to use, (tetramethylbenzidine) TMB.
- **Stop Solution - symbol** STOP 1 vial, 6.0 mL of 1 mol/L sulfuric acid.
- **Wash Solution Concentrate - symbol** WASH 40X 1 vial, 25 mL (40X concentrated), PBS-Tween wash solution.
- **IFU:** 1 copy.
- **Plate Lid**: 2 pieces.

Materials required (but not provided)
- Microplate reader with 450nm and 620nm wavelength absorbent capability.
- Microplate washer.
- Incubator.
- Plate shaker.
- Micropipettes and multichannel micropipettes delivering 50µl with a precision of better than 1.5%.
- Absorbent paper.
- Distilled water

Precautions and warnings
- Exercise the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.
- Do not use reagents beyond the labeled expiry date.
- Do not mix or use components from kits with different batch codes.
- All the specimen and reaction wastes should be considered potentially biohazard. The handling of specimens and reaction wastes should be in accordance with the local regulations and guidelines.
- The Stop Solution contains sulfuric acid, which can cause severe burns. In the event of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Neutralized acids and other liquid waste should be decontaminated by adding a sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. Exposure to 1.0% sodium hypochlorite for 30 minutes may be necessary to ensure effective decontamination.
- Some reagents contain 0.05% or 0.1% ProClin 300 which may cause sensitization by skin contact, which must therefore be avoided. Reagents and their containers must be disposed of safely. If swallowed, seek medical advice immediately and show this container or label.
- Substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- For information on hazardous substances included in the kit please refer to the Materials Safety Data Sheet (MSDS), which is available on request.
- Do not smoke, drink, eat or apply cosmetics in the work area.
- Do not pipette by mouth. Wear protective clothing, disposable gloves and eye/face protection when handling samples and reagents. Wash hands after use.
- If any of the reagents comes into contact with the skin or eyes, wash the area extensively with water.
- **Indications of instability deterioration of the reagent:** Values of the Positive or Negative controls, which are out of the indicated quality control range, are indicators 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 and proven deterioration or instability of the reagents, immediately substitute the reagents with new one.

Storage and stability
- Store at 2-8℃.
- Don't freeze.
- Seal and return unused reagents to 2-8℃, under which conditions the stability will be retained for 2 months, or until the labeled expiry date, whichever is earlier.

Specimen collection and preparation
- Human serum or plasma is recommended for this assay.
- Cap and store the samples at 18-25 ℃ for no more than 8 hours. Stable for 7 days at 2-8℃, and 1 month at -20℃. Freeze only once.
- Do not use heat-inactivated samples.
- Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation.
- Avoid grossly hemolytic, lipemic or turbid samples.

Wash solution (40X dilution)
Add deionized water to the 40X concentrated Wash Solution Concentrate.
Dilute 25 mL of Wash Solution Concentrate with 975 mL of deionized water to a final volume of 1000 mL. Stable for 2 months at room temperature.

Test procedure
Ensure the patients' samples and reagents are at ambient temperature (18-25 ℃) before measurement. Mix all reagents through gently inverting prior to use.
- **Preparation:** Mark three wells as Negative control (e.g. B1, C1, D1), two wells as Positive control (e.g. E1 for HIV-1 and F1 for HIV-2) and one Blank (e.g. A1, neither samples nor HRP-Conjugate should be added into the Blank well).
- **Adding Sample**: Add **100 µL** of **Positive control, Negative control,** and **Specimen** into their respective wells except the Blank, mix by tapping the plate gently.
  Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination.
- **Incubation:** Cover the plate with the plate cover and incubate for **60 minutes at 37℃.**
- **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Washing buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
- **Adding Conjugate:** Add **100 µL** of **Conjugate** into each well except the Blank, and mix by tapping the plate gently.
- **Incubation:** Cover the plate with the plate cover and incubate for **30 minutes at 37℃**.
- **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Washing buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.
- **Coloring:** Add **100 µL** of **Substrate** into each well including the Blank. **Incubate** for **10 minutes** at room temperature avoiding light. The enzymatic reaction between the substrate solutions and the Conjugate produces blue color in Positive control and Anti-HIV1/2 positive sample wells.
- **Stopping:** Using a multichannel pipette or manually, add **50 µL** of **Stop Solution** and mix gently. Intensive yellow color develops in Positive control and HIV1/2 positive sample wells.
- **Measuring:** 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 10 minutes after stopping the reaction

Quality Control and calculation of the 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 specimen absorbance (A) value to the Cut-off value (C.O.) of the plate. The results should be calculated by subtracting the Blank well A value from the print report values of specimens and controls.

Calculation of the Cut-off value (C.O.) = NC+0.12
(NC = the mean absorbance value for three negative controls).
Quality control (assay validation)
The test results are valid if the Quality Control criteria are fulfilled. 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.

Anti-HIV1/2

diasino

Antibodies to human immunodeficiency virus 1/2 (ELISA)

- The A value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450 nm.
- The A values of the Positive control must be ≥ 0.800 at 450/630nm or at 450nm after blanking.
- The A values of the Negative control must be < 0.100 at 450/630nm or at 450nm after blanking.

If one of the Negative control A values does not meet the Quality Control criteria, it should be discarded and the mean value calculated again using the remaining value. If more than one Negative control A values do not meet the Quality Control Range specifications, the test is invalid and must be repeated.

Example:

1. Quality Control
Blank well A value: A1= 0.025 at 450nm

Well No.:	**B1**	**C1**	D1
Negative control A values after blanking:	0.020	0.012	0.016
Well No.:	**E1**	**F1**	
Positive control A values after blanking:	2.421	2.369	

All control values are within the stated quality control range

2. Calculation of Nc: $= \frac{(0.020 + 0.012 + 0.016)}{3} = 0.016$

3. Calculation of the Cut-off: (C.O.) = 0.016 + 0.12 = 0.136

Interpretation of results

Negative Results (A/C.O. < 1)

Specimens giving absorbance less than the Cut- off value are negative for this assay, which indicates that no Anti-HIV1/2 has been detected with this ELISA kit. Therefore, the patient is probably not infected with Anti-HIV1/2 and the blood unit do not contain antibodies to HIV 1/2 and could be transfused in case that other infectious disease markers are also absent.

Positive Results (A/C.O. ≥ 1)

Specimens giving an absorbance equal to or greater than the Cut-off value are considered initially reactive, which indicates that Anti-HIV1/2 has probably been detected using this ELISA kit. All initially reactive specimens should be retested in duplicates using this ELISA kit before the final assay results interpretation. Repeatedly reactive specimens could be considered positive for Anti-HIV1/2 and therefore there are serological indications for infection with Anti-HIV1/2.

Borderline (A/C.O. = 0.9-1.1)

Specimens with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results.

- If, after retesting of the initially reactive samples, both wells are negative results (A/C.O.<0.9), these samples should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step.
- If after retesting in duplicates, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for antibodies to HIV 1/2 and therefore the patient is probably infected with HIV1/2 and the blood unit must be discarded.
- After retesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.

Performance characteristics

Specificity

A total of 2215 fresh serum and plasma samples from volunteer blood donors and medical examination donors were collected and tested at different geographically distinct blood centers and hospitals, 7 samples were repeatedly reactive, and based on supplemental test results from a licensed and/or research immunoblot assay, they were Anti-HIV negative. Thus this assay has a 99.68% (2208/2215) specificity.

Sensitivity

The sensitivity of this assay was determined by testing a panel of 420 positive samples, all samples resulted positive, therefore the diagnostic sensitivity was 100%.

Limitations and interferences

- This reagent kit is to be used for un-pooled human serum or plasma only.
- Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
- Specimens with very low level of HIV/1/2 Ab may not consistently repeat positive. In this case, it is recommended to test follow-up samples.
- Anti-HIV1/2 negative result does not preclude the possibility of infection with HIV1/2.
- Non-repeatable false positive results may occur due to non-specific binding of the sample and conjugate to the wall of the well(s).

References

1. Barré-Sinoussi F, Chermann JC, Rey F, et al. Isolation of a T-lymphotropic Retrovirus from a Patient at Risk for Acquired Immune Deficiency Syndrome (AIDS). Science 1983;220:868-871.
2. Popovic M, Sarngadharan MG, Read E, et al. Detection, Isolation and Continuous Production of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and Pre-AIDS. Science 1984;224:497-500.
3. Gürtler LG, Hauser PH, Eberle J, et al. A New Subtype of Human Immunodeficiency Virus Type 1 (MVP-5180) from Cameroon. J Virol 1994;68(3):1581-1585.
4. Simon F, Mauclère P, Roques P, et al. Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. Nat Med 1998;4(9):1032-1037.
5. Robertson DL, Anderson JP, Bradac JA, et al. HIV-1 nomenclature Proposal. Science 2000;288(5463):55-56.
6. Gürtler LG. Difficulties and strategies of HIV diagnosis. Lancet 1996;348:176-179.

Symbols

IVD	8°C / 2°C		REF
In vitro diagnostic medical device	**Temperature limit**	**Consult instructions for use**	**Catalog number**
LOT			Σ
Batch code	**Date of manufacture**	**Use-by date**	**Contains sufficient for <n> tests**
Manufacturer	**Do not use if package is damaged and consult instructions for use**		

diasino

DiaSino Laboratories Co., Ltd
No.68 Jingnansi Road National Eco & Tech Development Area
Zhengzhou 450000, P.R. China. info@diasino.com