AccuDiag™ HIV 1, 2 ELISA

Cat# 1516-12

IVD See external Label 2°C 8°C Σ 96 Tests

Test	HIV 1, 2
Method	Enzyme Linked Immunosorbent Assay
Principle	Double antigen Sandwich ELISA
Detection Range	Qualitative Positive; Negative & Cut off Calibrators
Sample	100 µL serum
Total Time	~ 75 min
Shelf Life	12-13 Months from the manufacturing date
Specificity	99.89%
Sensitivity	99.95%

INTENDED USE

The **HIV 1, 2 ELISA Test** is an enzyme-linked immunosorbent assay which is used for the qualitative detection of antibodies to HIV (human immunodeficiency virus) type 1 (group M-O) or type 2 in human serum/plasma. This HIV ELISA can best be used as a diagnostic aid in detecting HIV-1 and HIV-2 infections (etiological agents of AIDS) and/or for blood donor screening.

SUMMARY AND EXPLANATION

Testing for the presence of HIV antigens or antibodies from serum is the best way to obtain serological evidence of infection from individuals that may be harboring the HIV virus. Detection of antigen can be evidenced in both acute phase and symptomatic phases of AIDS. However, the HIV-1 and HIV-2 antibodies can be identified all through the entire infection period - beginning at the acute phase all the way through the last stages of AIDS. For the serodiagnosis of HIV infection, the best approach is use of highly sensitive antibody ELISA tests. The predominant path of HIV infection is blood transfusion (independent of the other dominant carrier - sexual transmission). All donated blood or plasma is tested because HIV can be present in both cellular and cell-free fractions of human blood. This creates a high risk transmission rate if not tested properly.

TEST PRINCIPLE

Polystyrene microwell strips pre-coated with recombinant HIV antigens indicated in E.coli (recombinant HIV-1gp41, gp120, and recombinant HIV-2 gp-36) make up this two-step incubation antigen sandwich enzyme immunoassay kit (HIV-1 & 2 ELISA). At the time of the first incubation step, patient's plasma or serum sample is added. If specific HIV1/2 antibodies are present, they will be captured inside the wells. After this, microwells are washed to remove unbound serum proteins. Added after this stage is the second set of recombinant antigens conjugated to the enzyme Horseradish Peroxidase (HRP-Conjugate) and indicating the same epitopes as the pre-coated antigens. While the second incubation occurs, these antigens will bind to the captured antibody. The chromogen solutions can be added to the wells, but not before the microwells are washed again to remove unbound conjugate. In the wells where the antigen-antibody-antigen (HRP) sandwich immunocomplex is taking place, the colorless chromogens are hydrolyzed by the bound HRP conjugate to a blue-colored product. After the reaction is stopped with sulfuric acid, the blue color turns yellow.

What can be measured at this point is the amount of color intensity proportional to the amount of antibody captured in the wells, and to the sample. Colorless wells indicate negative for anti-HIV 1/2.

Assay principle scheme: Double antigen sandwich ELISA

Ag(p)+Ab(s) → [Ag(p)–Ab(s)+ENZ] → [Ag(p)-Ab(s)–ENZ] → blue→yellow (+)
Ag(p) → [Ag(p) +ENZ] → [Ag(p)] → no color (-)

Incubation1	Incubation2	Immobilized Complex	Coloring	Results
30 min	30min.		15min.	

Ag (p)–pre-coated recombinant HIV 1/2 antigens;
Ab(s)–HIV antibodies in sample;
ENZ–HRP conjugated recombinant HIV1/2 antigens;

SPECIMEN COLLECTION AND PREPARATION

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 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Do not heats 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.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. Σ 96 test
2. **MICROWELL PLATE** 1plate
   Blank microwell strips fixed on white strip holder. The plate is sealed in aluminium pouch with desiccant. 8 X 12/12 X 8-well strips 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.
3. **NEGATIVE CONTROL** 1vial
   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 2-8°C.
4. **POSITIVE CONTROL SERUM-1 (HIV 1)** 1vial
   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.

5. **POSITIVE CONTROL SERUM-2 (HIV 2)** 1vial
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.
6. **HRP-CONJUGATE REAGENT** 1vial
Red-colored liquid filled in a white vial with red/orange screw cap.
13 ml per vial.
Horseradish peroxidase-conjugated HIV 1/2 antigens.
Ready to use as supplied. Once open, stable for one month at 2-8°C.
7. **WASH BUFFER** 1bottle
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 concentration 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.
8. **CHROMOGEN SOLUTION A** 1vial
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
9. **CHROMOGEN SOLUTION B** 1vial
Colorless liquid filled in a brown vial with brown/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
10. **STOP SOLUTION** 1vial
Colorless liquid filled in a white vial with yellow screw cap.
8ml per vial.
Diluted sulfuric acid solution (2.0M H_2SO_4).
11. **PLASTIC SEALABLE BAG** 1unit
For enclosing the strips not in use.
12. **CARDBOARD PLATE COVER** 2sheets
To cover the plates during incubation and prevent evaporation or contamination of the wells.
13. **PACKAGE INSERTS** 1copy

Materials 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.

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.
7. The concentrated Washing solution should be diluted **1 to 2** before use. For one plate, mix 50 ml 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.

ASSAY PROCEDURE

1. **Reagents preparation:** Allow the reagents and samples to reach room temperature **(18-30°C)** for at least 15-30minutes**.** Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, 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.
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.
3. **Adding Samples:** Add **100**µl 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).**
4. **Incubation (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.
5. **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well 5times 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.
6. **Adding HRP-Conjugate:** Add **100µl** HRP- Conjugate into each well except in the Blank.
7. **Incubation (2):** Cover the plate with the plate cover and incubate for **30minutes at 37°C**.
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**.
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 15minutes 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.
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.

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)

RESULTS

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.

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	00.32	0.031	0.027

Nc = 0.030
2.**Calculation of cut-off (C.O)** = 0.030 + 0.12 = 0.150

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

- **Negative(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, therefore the patient is probably not infected or the blood unit do not contain antibodies to HIV ½ and could be transfused.
- **Positive (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. Repeatedly reactive samples could be considered positive for antibodies to HIV 1/2 and therefore the patient is probably infected with HIV 1/2. Any blood unit containing antibodies to HIV 1/2 should be immediately discarded.
  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. Repeatedly positive samples could be considered positive for antibodies to HV 1/2. Follow up and supplementary testing of any positive samples with other analytical system (e.g. WB, PCR) is required before establishing of the final diagnosis.

PERFORMANCE CHARACTERISTICS

The **clinical performances** of this HIV 1+2 ELISA have been evaluated by a panel of samples obtained from 11045 healthy blood donors in 10 testing centers and by a panel of samples from 783 HIV 1/2 positive patients -757 HIV -1, 7 HIV-2, 2 HIV-O and 17 HIV-1(M). The positive samples have been characterized based upon the individual patient CD4+ count and/or Western Blot (WB) or NAT. Results obtained in individual laboratories may differ.

Analytical specificity:
No cross reactivity was observed with specimens from patients infected with HAV, HCV, HBV, HTLV, CMV, and TP.No high dose hook effect and rheumatoid factor interference observed during clinical testing. The assay performance characteristics are unaffected from elevated concentrations of bilirubin, hemoglobin, and triolein. Frozen specimens have been tested to check for interferences due to collection and storage.

Specimen	No	-	+	- (WB)	Specificity
Donors	11045	11033	12	12	99.89%

Specimen	No	+	-	+(WB)	Sensitivity
HIV-1	757	756	1	757	99.86%
HIV-2	7	7	0	7	100%
HIV-O (BBI)	2	2	0	NA	100%

HIV - 1 Subtypes	No. samples	Result	WB
A	1	+	+
B	1	+	+
C	9	+ (9/9)	+
B'+C	4	+ (4/4)	+
E	1	+	+
G	1	+	+

Test Performance on Seroconversion Panels (BBI)

ID#	Bleed days	HIV	Comparative HIV EIAs					Western Blot
PRB917								
01	0	-	-	-	-	-	-	vf24
02	53	-	-	-	-	-	-	vf24
03	57	-	-	-	-	-	-	vf24
04	60	+	+	-	-	-	+	vf24
05	65	+	+	-	-	+	+	vf24
06	67	+	+	-	-	+	+	vf24
07	72	+	+	+	+	+	+	24,vf41, vf160
PRB918								
01	0	-	-	-	-	-	-	No bands
02	2	+	+	-	-	-	-	No bands
03	7	+	+	-	-	+	+	Vf24
04	13	+	+	+	+	+	+	24,160
05	15	+	+	+	+	+	+	24,160
06	21	+	+	+	+	+	+	24,160,vf65

Reproducibility		Within run		Between run	
Specimen Type	No tests	Mean OD	CV%	Mean OD	CV%
Weak positive	10	0.415	6.2%	0.404	6.5%
Moderate positive	10	1.151	4.8%	1.012	5.3%
Strong positive	10	2.710	4.0%	2.695	4.3%
Positive control	10	2.361	4.1%	2.351	4.3%

QUALITY CONTROL

If one of the Negative control values does not meet the Quality control range specifications, it should be discarded and the mean value is calculated 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.

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 equal to or less than 0.100 at 450/630nm or at 450nm after blanking.

LIMITATIONS OF PROCEDURE

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. Any positive results should be confirmed with another available method and interpreted in conjunction with the patient clinical information.
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. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
7. The assay cannot distinguish between infections with HIV-1 and HIV-2.
8. This is a qualitative assay and the results can not 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.

PRECAUTIONS

This kit is intended **FOR IN VITRO USE ONLY.**
FOR PROFESSIONAL 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 micro well 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 ½, 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 1 hour 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 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-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalins 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.

STORAGE

1. 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 anti-HIV 1 + 2 ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.
2. **Please do not use this kit beyond the expiry date indicated on the kit box and reagent labels.**

SUMMARY OF THE ASSAY PROCEDURE:	
Add sample	**100 µl**
Incubate	**30 minutes**
Wash	**5times**
Add HRP-Conjugate	**100µl**
Incubate	**30 minutes**
Wash	**5 times**
Coloring	**50µl A + 50µl B**
Incubate	**15 minutes**
Stop the reaction	**50µl stop solution**
Read the absorbance	**450nm or 450/630 nm**

ISO 13485
ISO 9001

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