AccuDiag™ HIV 1&2 Ag/Ab ELISA Kit

REF 1520-P1

IVD See External Label 2°C-8°C Σ 96 Tests

HIV 1&2 Ag/Ab ELISA	
Principle	Sandwich ELISA
Detection	Qualitative
Sample	100 μL serum/plasma
Incubation Time	105 minutes
Sensitivity	100%
Specificity	100%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The DAI HIV 1+2 Ag/Ab ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for qualitative detection of antigens and/or antibodies to Human Immunodeficiency Viruses (HIV) type 1 (group M - O) and/or type 2 in human serum or plasma samples. The method is also known as 4th generation ELISA for HIV detection. The kit is intended for screening of blood donors and as an aid in the diagnosis of clinical conditions related to infection with HIV-1 and/or HIV-2 - the etiological agents of the acquired immunodeficiency syndrome (AIDS).

SIGNIFICANCE AND 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. 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. Therefore, all donations of blood or plasma should be tested due to the risk of HIV transmission through contaminated blood.

The ELISA tests for detection of HIV infection are characterized with high sensitivity, specificity and simple operation procedure. There are most appropriate for testing of large numbers of specimens and currently, internationally available are hundreds of HIV tests used in routine blood screening or clinical diagnosis. 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 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.

ASSAY PRINCIPLE

DAI 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.

SPECIMEN COLLECTION & PREPARATION

1. **Specimen Collection:** No special patient's preparation required. Collect the specimen in accordance with the normal laboratory practice. Either fresh serum or plasma specimens can be used with 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 haemolysis of the RBC. Care should be taken to ensure that the serum specimens are clear and not contaminated by microorganisms. Any visible particulate matters in the specimen should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature or by filtration.
2. Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, but ***highly lipaemic, icteric, or hemolytic specimens should not be used*** as they can give false results in the assay. ***Do not heat inactivate specimens***. This can cause deterioration of the target analyte. Samples with visible microbial contamination should never be used.
3. DAI HIV 1+2 Ag/Ab ELISA is intended ONLY for testing of individual serum or plasma samples. Do not use the assay for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
4. **Transportation and Storage:** Store specimens at 2-8°C. Specimens not required for assaying within 1 week 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 transportation of clinical samples and ethological agents.

REAGENTS

Materials provided with the kit

1. **MICROWELL PLATE:** Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. Each well contains recombinant HIV 1/2 antigens and anti-p24 antibodies. The microwell strips can be broken to be used separately. Place unused strips in the provided plastic storage bag together with the desiccant and return to 2-8°C. Once opened, the plate strips are stable for 4 weeks when stored at 2-8°C together with the desiccant. The microwell strips are for SINGLE USE only. Do not use if the vacuum sealing has been damaged when first time taken of out the box.
2. **NEGATIVE CONTROL:** Yellow-colored liquid filled in a vial with green screw cap.Protein-stabilized buffer tested non-reactive for HBsAg and antibodies to HIV 1/2, HCV, TP. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
3. **POSITIVE CONTROL-1:** Red-colored liquid filled in a vial with red screw cap.Protein-stabilized buffer solution tested positive for antibodies to HIV-1.Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
4. **POSITIVE CONTROL-2:** Red-colored liquid filled in a vial with yellow screw cap. Protein-stabilized buffer solution tested positive for antibodies to HIV-2. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
5. **POSITIVE CONTROL-Ag:** Red-colored liquid filled in a vial with blue screw cap. Protein-stabilized buffer solution tested positive for HIV p24 recombinant antigen. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
6. **HRP-CONJUGATE:** Red-colored liquid in a white vial with red screw cap. Horseradish peroxidase-conjugated recombinant HIV 1+2 antigens. Horseradish peroxidase conjugated avidin. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
7. **BIOTIN-CONJUGATE:** Blue-colored liquid in a white vial with blue screw cap. Biotinylated anti-HIV p24 antibodies diluted in protein-stabilized buffer. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
8. **WASH BUFFER:** Colorless liquid filled in a clear bottle with white screw cap. Buffer solution containing surfactant. The concentrate must be diluted **1 to 20** with distilled/ deionized water before use. Once diluted, stable for 1 week at room temperature, or for 2 weeks when stored at 2-8°C.
9. **CHROMOGEN SOLUTION A:** Colorless liquid filled in a white vial with green screw cap. Urea peroxide solution. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
10. **CHROMOGEN SOLUTION B:** Colorless liquid filled in a black vial with black screw cap. TMB (Tetramethyl benzidine), N,N-dimethylformamide. Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.
11. **STOP SOLUTION:** Colorless liquid in a white vial with yellow screw cap. Diluted sulfuric acid solution (0.5M H_2SO_4). Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.

- **PLASTIC SEALABLE BAG:** For enclosing the strips not in use 1unit
- **PACKAGE INSERT** 1copy

- **CARDBOARD PLATE COVER**
  3 sheets

To cover the plates during incubation and prevent evaporation or contamination of the wells.

Materials required but not provided

Freshly distilled or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing system and/or pipette, disposable pipette tips, absorbent tissue or clean towel, dry incubator or water bath, 37±1°C, plate reader, single wavelength 450nm or dual wavelength 450/600~650nm, microwell aspiration/wash system.

REAGENT PREPARATION

Allow the reagents to reach room temperature **(18-30°C)**. 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 (20X) as indicated in the instructions for washing. Use distilled or deionized water and only clean vessels to dilute the buffer. All other reagents are **READY TO USE AS SUPPLIED**.

Preparation: Mark three wells as Negative control **(e.g. B1, C1, D1)**, three wells as Positive control **(e.g. E1 for HIV-1, F1 for HIV-2 and G1 for HIV-Ag)** and one Blank (**e.g. A1**, neither specimens nor 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 for the test.

Adding Biotin-Conjugate: Add **20µl** of Biotin-Conjugate into each well except the Blank.

Adding Specimen: Add **100µl** of Positive Controls, Negative Controls, and Specimens into the irrespective wells except the Blank, mix gently. **Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination.**

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

Washing: 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 down the plate onto blotting paper or clean towel, and tap it to remove any remainders.

Adding HRP-Conjugate: Add **100µl** of HRP-Conjugate into each well except the Blank.

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

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 **50µl** of Chromogen Solution A and then **50µl** of Chromogen Solution B into each well including the Blank, mix 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 for antigens / antibodies specimen wells.

Stopping Reaction: Using a multichannel pipette or manually, add **50µl** of Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and HIV 1/2 positive for antigens / antibodies specimen wells.

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 600~650nm**. Calculate the Cut-off value and evaluate the results.
(***Note: read the absorbance within 10 minutes after stopping the reaction***).

Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

Add Biotin-Conjugate	20µl
Add Samples	100µl
Incubate	60minutes
Wash	5times
Add HPR-Conjugate	100µl
Incubate	30minutes
Wash	5times
Coloring	50µl A + 50µl B
Incubate	15minutes
Stop the reaction	50µl stop solution
Read the absorbance	450nm or 450/600~650 nm

INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential in order 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 specimen or HRP-conjugate, after incubation, do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. 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 washing 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 they are wasted in an appropriate way.
7. The concentrated Wash buffer should be diluted **1:20** before use. If less than a whole plate is used, prepare the proportional volume of solution.

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. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well A value from the print report values of specimens and controls. In case the reading is based on dual filter plate reader, do not subtract 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).

INTERPRETATION

Negative Results (A / C.O. < 1): Specimens 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 DAI HIV 1+2 Ag/Ab ELISA, therefore the patient is probably not infected with HIV 1/2 and the blood unit do not contain antibodies to HIV 1/2 or p24 antigen and could be transfused in case that other infectious diseases 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 HIV 1/2 antibodies and/or p24 antigen have probably been detected using DAI HIV 1+2 Ag/Ab ELISA. All initially reactive specimens should be retested in duplicates using DAI HIV 1+2 Ag/Ab ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for antibodies to HIV 1/2 and/or p24 antigen with DAI HIV 1+2 Ag/Ab ELISA.

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.

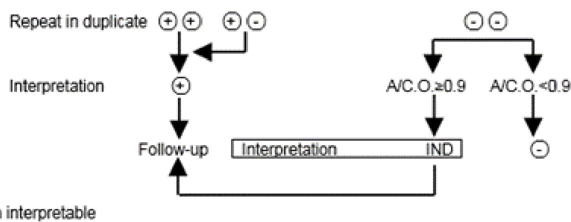
Follow-up, confirmation and supplementary testing of any positive specimen with other analytical system (e.g. WB, PCR) is required. Clinical diagnosis should not be established based on a single test result. It should integrate clinical and other laboratory data and findings.

INITIAL RESULTS

INTERPRETATION AND FOLLOW-UP

ALL INITIALLY

REACTIVE OR BORDERLINE SPECIMENS



- 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. For more information regarding DAI's ELISA Troubleshooting, please refer to DAI's "ELISAs and Troubleshooting Guide".
- 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/or p24 antigen, therefore the patient is probably infected with HIV 1/2 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.

QUALITY CONTROL

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.

- 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 450/600~650nm or at 450nm after blanking.
- The A values of the Negative control must be ≤ 0.100 at 450/600~650nm 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 two values. 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 (Note: blanking is required only when reading with single filter at 450nm)

Well No.:	**B1**	**C1**	**D1**
Negative control A values after blanking:	0.020	0.012	0.016
Well No.:	**E1**	**F1**	**G1**
Positive control A values after blanking:	2.421	2.369	2.893

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

PERFORMANCE CHARACTERISTICS

In a performance evaluation study, DAI HIV 1+2 Ag/Ab ELISA demonstrated sensitivity in detection of HIV infection of 100%. The demonstrated specificity was of 99.76%.

Samples Type	Samples No.	+	-	Positive (WB)	Specificity	Sensitivity
Healthy Blood Donors	2968	7	2961	0	99.76%	——
High Risk Population	1462	181	1281	178	99.76%	100%

Multi-center performance evaluation study was organized to access the performance characteristics of DAI HIV 1+2 Ag/Ab ELISA demonstrated

sensitivity in detection of HIV infection of 100%. The demonstrated specificity was of 99.75%.

Site	Samples	No.	DAI HIV 1+2 Ag/Ab ELISA +	-
Blood bank	-	2685	2	2683
Hospital	+	297	297	0
	-	203	0	203
Blood bank	+	120	120	0
	-	4860	12	4848
Ref. lab	+	132	132	0
	-	1011	5	1006
Hospital	+	128	128	0
	-	72	3	69
Total	+	677	Sensitivity 100%	
	-	8831	Specificity 99.75%	

The performance characteristics of DAI HIV 1+2 Ag/Ab ELISA were compared with three other commercially available kits for the detection of HIV antibodies or HIV antigen and antibodies. Overall agreement of 99.89-100% was demonstrated.

	DAI 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-2 (HIV-Ab)	+	2	1	3
	-	0	2682	2682
	TOTAL	2	2683	2685
AGREEMENT: (2+2682)/2685 = 99.96%				
		+	-	TOTAL
EIA-3 (HIV-Ag/Ab)	+	0	1	1
	-	2	2682	2684
	TOTAL	2	2683	2685
AGREEMENT: (0+2682)/2685 = 99.89%				

After testing of more than 15000 negative and 700 HIV positive samples, the specificity of DAI HIV 1+2 Ag/Ab ELISA was calculated as over 99.80%, and the demonstrated sensitivity was 100%.

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.

LIMITATIONS OF THE ASSAY

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antibodies or p24 antigen may be undetectable during the early stage of the disease and in some immunosuppressed individuals. Therefore, negative results obtained with DAI HIV 1+2 Ag/Ab ELISA are only indication that the specimen does not contain detectable level of HIV 1/2 antibodies or p24 antigen and any negative result should not be considered as conclusive evidence that the individual is not infected with HIV 1/2 or the blood unit is not infected with HIV 1/2.
3. If, after retesting of the initially reactive specimens, the assay results are negative, these specimens 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. For more information regarding Diagnostic Automation's ELISA Troubleshooting, please refer to Diagnostic Automation's "ELISAs and Troubleshooting Guide", or contact Diagnostic Automation's technical support for further assistance.
4. The most common assay mistakes are: using kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolyzed specimens or specimens containing fibrin, incompletely clotted serum specimens.
5. The prevalence of the marker will affect the assay's predictive values.
6. This assay cannot be utilized to test pooled (mixed) serum or plasma. DAI HIV 1+2 Ag/Ab ELISA has been evaluated only with individual serum or plasma specimens.
7. DAI HIV 1+2 Ag/Ab ELISA is a qualitative assay and the results cannot be used to measure antibody or antigen concentration. This assay cannot distinguish between infections with HIV-1 and HIV-2. This assay cannot distinguish between positive antibody and positive p24 antigen results.

STORAGE CONDITIONS

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 DAI HIV 1+2 Ag/Ab ELISA, during storage, protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS

TO BE USED ONLY FROM QUALIFIED PROFESSIONALS
The ELISA assays are time and temperature sensitive. 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 for optimal performance of the tests.
2. Make sure that all reagents are within the validity indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels or boxes.
3. CAUTION - CRITICAL STEP: Allow the reagents and specimens to reach room temperature (18-30°C) before use. Shake reagent gently before use. Return at 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 the reading. When reading the results, ensure that the plate bottom is dry and there are no air bubbles inside the wells.
6. 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.
7. Avoid assay steps long time interruptions. Assure same working conditions for all wells.

8. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations.
9. Assure that the incubation temperature is 37°C inside the incubator.
10. When adding specimens, do not touch the well's bottom with the pipette tip.
11. When measuring with a plate reader, determine the absorbance at 450nm or at 450/630nm.
12. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.
13. If using fully automated equipment, 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.
14. All specimens from human origin should be considered as potentially infectious. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety.

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 specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Bovine derived sera have been used for stabilizing of the positive and negative controls. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.

15. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
16. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
17. The pipette tips, vials, strips and specimen 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. Materials Safety Data Sheet (MSDS) available upon request.
18. Some reagents may cause toxicity, irritation, burns or have carcinogenic effect as raw materials. Contact with the skin and the mucosa should be avoided but not limited to the following reagents: Stop solution, the Chromogens, and the Wash buffer.
19. The Stop solution 0.5M H_2SO_4 is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with the skin or eyes.
20. ProClin™ 300 0.1% used as preservative, can cause sensation of the skin. Wipe up spills immediately or wash with water if come into contact with the skin or eyes.

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 or contact DACD technical support for further assistance.

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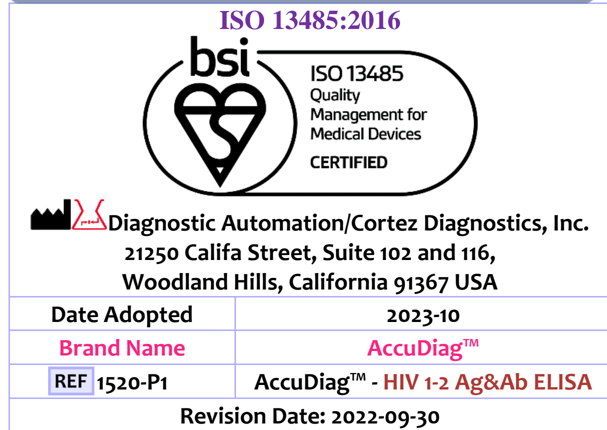
MANUFACTURER AND BRAND DETAILS



Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

Date Adopted	2023-10
Brand Name	AccuDiag™
REF 1520-P1	AccuDiag™ - HIV 1-2 Ag&Ab ELISA
Revision Date: 2022-09-30	