WHO Prequalification of In Vitro Diagnostics Programme
PUBLIC REPORT

Product: AiD™ anti-HIV 1+2 ELISA
Number: PQDx 0006-005-00

Abstract

AiD™ anti-HIV 1+2 ELISA with product codes **WI-4396** and **WI-43480**, manufactured by **Beijing Wantai Biological Pharmacy Enterprise Co., Ltd**, **rest of world regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 15 February 2016.

Intended use:
AiD™ anti-HIV 1+2 ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for qualitative detection of antibodies to Human Immunodeficiency Viruses (HIV) type 1 (group M - O) or type 2 in human serum or plasma samples. The assay can be utilized for screening of blood donors and/or 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).

Assay description:
AiD™ anti-HIV 1+2 ELISA is a two-step incubation antigen "sandwich" enzyme immunoassay, which uses polystyrene microwell strips pre-coated with recombinant HIV antigens expressed in E.coli (recombinant HIV-1gp41, gp120, and recombinant HIV-2 gp-36). Patient specimen (serum or plasma) 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 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 specimens negative for anti-HIV 1/2 remain colorless.

Reactive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.

Test kit contents:

	96 tests (product code WI-4396)	**480 tests (product code WI-43480)**
Microwell Plate: Blank microwell strips fixed on white strip holder. The plate is sealed in aluminium pouch with desiccant. Each well contains recombinant HIV 1/2 antigens (recombinant HIV-1 gp41, gp120, and recombinant HIV-2 gp36). The microwell strips can be broken to be used separately.	1x 96 wells 12x 8 well plate	5x 96 wells 12x 8 configuration
Negative Control: Protein-stabilized buffer tested no- reactive for non-reactive for HBsAg, and antibodies to HIV 1/2, HCV and TP Yellow-colored liquid filled in a vial with green screw cap. Preservative 0.1% ProClin™ 300.	1x 1ml vial	3x 1ml vial
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. Preservative 0.1% ProClin™ 300.	1x 1ml vial	3x 1ml vial
Positive Control-2: Protein-stabilized buffer solution tested positive for antibodies to HIV-2. Red-colored liquid filled in a vial with yellow screw cap. Preservative 0.1% ProClin™ 300.	1x 1ml vial	3x 1ml vial
Hrp-Conjugate: Horseradish peroxidase-conjugated recombinant HIV 1+2 antigens. Red-colored liquid in a white vial with red screw cap. Preservative 0.1% ProClin™ 300.	1x 12ml vial	5x 12ml vial
Wash Buffer: Detergent Tween-20. Colorless liquid filled in a clear bottle with white screw cap. Buffer solution containing detergent. The concentrate must be diluted 1 to 20 with distilled/ deionized water before use. DILUTE BEFORE USE!	1x 50ml bottle	2x 125ml bottle
Chromogen Solution A: Urea peroxide solution. Colorless liquid filled in a white vial with green screw cap.	1x 8ml vial	1x 60ml vial
Chromogen Solution B: TMB solution (Tetramethyl benzidine). Colorless liquid filled in a black vial with black screw cap.	1x 8ml vial	1x 60ml vial
Stop Solution: Diluted sulfuric acid solution (0.5M H_2SO_4). Colorless liquid in a white vial	1x8ml vial	1x60ml vial

with yellow screw cap.		
Plastic Sealable Bag: For enclosing the strips not in use.	1	5
Instructions For Use	1	1
Cardboard Plate Cover	3	15

Storage:
The test kit should be stored at 2 to 8 °C.

Shelf-life:
15 months.

Summary of prequalification status for AiD anti-HIV 1+2 ELISA

	Initial acceptance	
	Date	**Outcome**
Status on PQ list	15 February 2016	listed
Dossier assessment	19 July 2013	MR
Inspection status	24 April 2015	MR
Laboratory evaluation	15 January 2014	MR

MR: Meets Requirements
NA: Not Applicable

AiD™ anti-HIV 1+2 ELISA was accepted for the WHO list of prequalified in vitro diagnostics on the basis of data submitted and publicly available information.

Background information

Beijing Wantai Biological Pharmacy Enterprise Co., Ltd submitted an application for prequalification of AiD™ anti-HIV 1+2 ELISA. Based on the established prioritization criteria, AiD™ anti-HIV 1+2 ELISA was given priority for prequalification.

Product dossier assessment

Beijing Wantai Biological Pharmacy Enterprise Co., Ltd submitted a product dossier for AiD™ anti-HIV 1+2 ELISA as per the Instructions for compilation of a product dossier (PQDx_018 v1). The information submitted in the product dossier was reviewed by WHO staff and external experts (assessors) appointed by WHO in accordance with the internal report on the screening and assessment of a product dossier (PQDx_009 v2). Based on the product dossier screening and assessment findings, a recommendation was made to accept the product dossier for AiD™ anti-HIV 1+2 ELISA for prequalification.

Commitments for prequalification: N/A

Manufacturing site inspection

A comprehensive inspection was performed at the site of manufacture (No. 31 Life Science Park Road Changping District, Beijing, China) of AiD™ anti-HIV 1+2 ELISA in December 2014 as per the Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics (PQDx_014 v1). The inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality. The manufacturer's responses to the nonconformities to the quality management system found at the time of the inspection were accepted 24 April 2015.

Commitments for prequalification: N/A

Laboratory evaluation

AiD™ anti-HIV 1+2 ELISA (Beijing Wantai Biological Pharmacy Enterprise, Co. Ltd.) was evaluated by WHO in the fourth quarter of 2012 using serum/plasma specimens. From this evaluation, we drew the following conclusions:

AiD™ anti-HIV 1+2 ELISA (Beijing Wantai Biological Pharmacy Enterprise, CO, Ltd.) is an enzyme immunoassay assay for the detection of HIV-1/2 antibodies in human serum/plasma. A volume of 100 µL of specimen is needed to perform the assay. This type of assay requires laboratory equipment and cannot be performed in laboratories with limited facilities. Reading of the results must be performed with a spectrophotometer.

In this limited evaluation on a panel of 1118 clinically-derived specimens, we found an initial sensitivity (95% CI) of 100% (99.2% - 100%) and an initial specificity (95% CI) of 99.09% (98.0% - 99.7%) compared to the reference assays. The final sensitivity (95% CI) was 100% (99.2% - 100%) and the final specificity (95% CI) was 99.54% (98.7% - 99.9%) compared to the reference assays. Lot to lot variation was acceptable.

For eight seroconversion panels, AiD™ anti-HIV 1+2 ELISA detected on average the same number of specimens as the benchmark assay; Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics). For the mixed titer panel, AiD™ anti-HIV 1+2 ELISA correctly classified 24 out of the 25 specimens. For the 1st International Reference Panel for anti-HIV [NIBSC code 02/210], AiD™ anti-HIV 1+2 ELISA correctly classified all specimens.
In this study, 0% of the results were recorded as indeterminate. The invalid rate was 0%.

Labelling

1. **Labels**
2. **Instructions for use**

1. Labels

Shipping box label

Beijing Wantai Biological Pharmacy Enterprise

Product Cat.No/ Name:

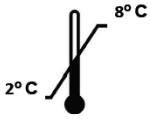
LOT:

EXP:

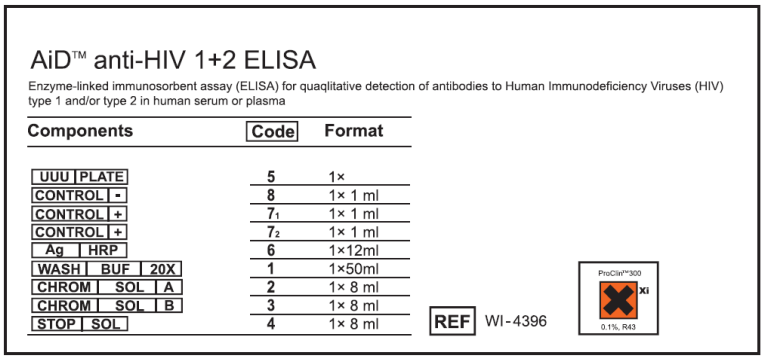
QUANTITY:

SHIP TO:

Carton No.



Kit box label for product code WI-4396



AiD™ anti-HIV 1+2 ELISA

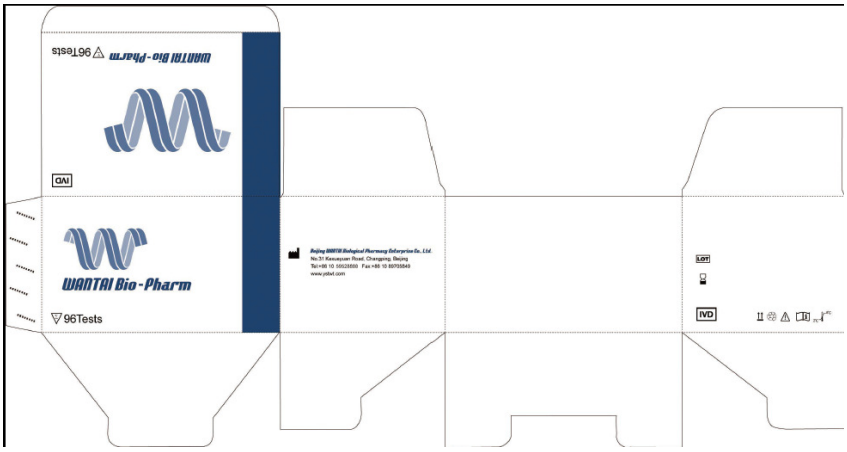
Enzyme-linked immunosorbent assay (ELISA) for quaqlitative detection of antibodies to Human Immunodeficiency Viruses (HIV) type 1 and/or type 2 in human serum or plasma

Components	Code	Format
UUU PLATE	5	1×
CONTROL -	8	1× 1 ml
CONTROL +	7_1	1× 1 ml
CONTROL +	7_2	1× 1 ml
Ag HRP	6	1×12ml
WASH BUF 20X	1	1×50ml
CHROM SOL A	2	1× 8 ml
CHROM SOL B	3	1× 8 ml
STOP SOL	4	1× 8 ml

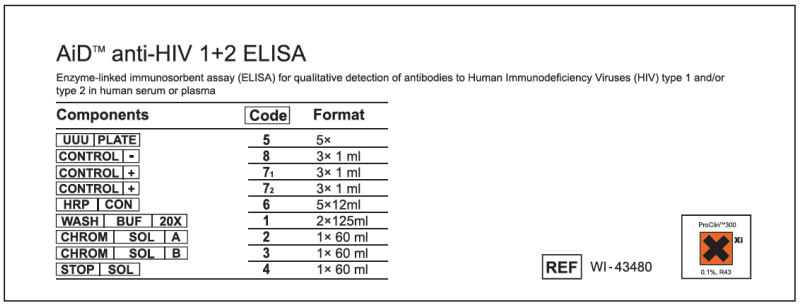
REF WI-4396

ProClin™300 Xi 0.1%, R43

Box label for product code WI-4396



Box label for product code WI-43480



AiD™ anti-HIV 1+2 ELISA

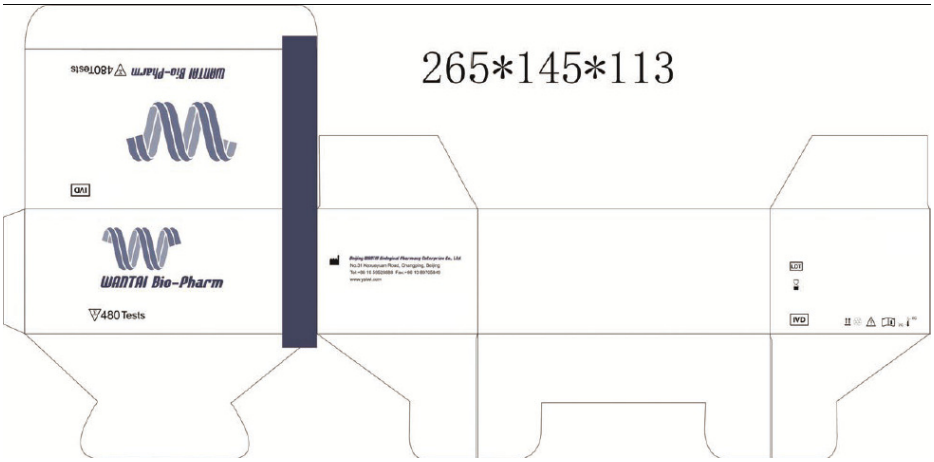
Enzyme-linked immunosorbent assay (ELISA) for qualitative detection of antibodies to Human Immunodeficiency Viruses (HIV) type 1 and/or type 2 in human serum or plasma

Components	Code	Format
UUU PLATE	5	5×
CONTROL -	8	3× 1 ml
CONTROL +	7_1	3× 1 ml
CONTROL +	7_2	3× 1 ml
HRP CON	6	5×12ml
WASH BUF 20X	1	2×125ml
CHROM SOL A	2	1× 60 ml
CHROM SOL B	3	1× 60 ml
STOP SOL	4	1× 60 ml

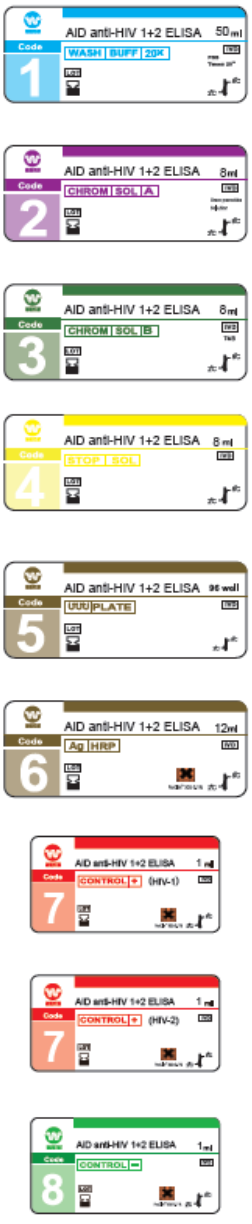
REF WI-43480

ProClin™300 XI 0.1%, R43

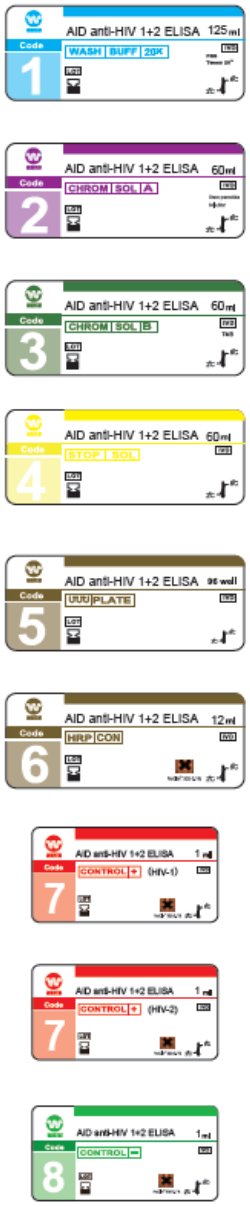
Box label for product code WI-43480



Component labels for product code WI-4396



Component labels for Product code WI-43480



Wantai Human Immunodeficiency Virus Diagnostics

AiD™ anti-HIV 1+2 ELISA

HUMAN IMMUNODEFICIENCY VIRUS (HIV) 1+2 ANTIBODY ELISA KIT

REF WI-4396 | V. 2015-01(10) [Eng.] | 96 Tests | IVD

Read the package insert carefully and completely before performing the assay. Follow the instructions and do not modify them. Only by strict adherence to these instructions, the erroneous results can be avoided and the optimal performance of AiD™ anti-HIV 1+2 ELISA achieved.

INTENDED USE

AiD™ anti-HIV 1+2 ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for qualitative detection of antibodies to Human Immunodeficiency Viruses (HIV) type 1 (group M - O) or type 2 in human serum or plasma specimens. The assay can be utilized for screening of blood donors and/or 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).

SUMMARY

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. Antigen 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[1]. Therefore, the use of highly sensitive antibody assays is the primary approach in serodiagnosis of HIV infection. 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[2]. This can be effectively achieved by testing for the antibodies to HIV-1 and HIV-2 by using a highly sensitive ELISA tests[3].

PRINCIPLE OF THE TEST

AiD™ anti-HIV 1+2 ELISA is a two step incubation antigen "sandwich" enzyme immunoassay kit, which uses 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 specimen 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 specimen respectively. Wells containing specimens non-reactive for anti-HIV 1/2 remain colorless.

IVD In Vitro Diagnostic Use Only

This kit contains reagents sufficient for testing of maximum of 91 specimens in a test run.

UUU | PLATE
Code 5 (1x96wells)
8×12/12×8-well per plate

MICROWELL PLATE: Blank microwell strips fixed on white strip holder. The plate is sealed in aluminium pouch with desiccant. Each well contains recombinant HIV 1/2 antigens (recombinant HIV-1 gp41, gp120, and recombinant HIV-2 gp36). The microwell strips can be separated to be used separately. Place unused strips in the provided plastic storage bag together with the desiccant and return to 2-8°C. Once open, 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.

CONTROL | -
Code 8 (1x1ml per vial)
preserv.0.1% ProClin™ 300

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 and TP. Ready to use as supplied. Once open, stable for 4 weeks at 2-8°C.

CONTROL | +
Code 7_1 (1x1ml per vial)
preserv.0.1% ProClin™ 300

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 open, stable for 4 weeks at 2-8°C.

CONTROL | +
Code T_2 (1x1ml per vial)
preserv.0.1% ProClin™ 300

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 open, stable for 4 weeks at 2-8°C.

Ag | HRP
Code 6 (1x12ml per vial)
preserv.0.1% ProClin™ 300

HRP-CONJUGATE: Red-colored liquid in a white vial with red screw cap. Horseradish peroxidase-conjugated recombinant HIV 1+2 antigens. Ready to use as supplied. Once open, stable for 4 weeks at 2-8°C.

WASH | BUF | 20X
Code 1 (1x50ml per bottle)
DILUTE BEFORE USE!
detergent Tween-20

WASH BUFFER: Colorless liquid filled in a clear bottle with white screw cap. Buffer solution containing detergent. 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.

CHROM | SOL | A
Code 2 (1x8ml per vial)

CHROMOGEN SOLUTION A: Colorless liquid filled in a white vial with green screw cap. Urea peroxide solution. Ready to use as supplied. Once open, stable for 4 weeks at 2-8°C.

CHROM | SOL | B
Code 3 (1x8ml per vial)

CHROMOGEN SOLUTION B: Colorless liquid filled in a black vial with black screw cap.TMB (Tetramethyl benzidine) solution. Ready to use as supplied. Once open, stable for 4 weeks at 2-8°C.

STOP | SOL
Code 4 (1x8ml per vial)

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 open, stable for 4 weeks at 2-8°C.

- **PLASTIC SEALABLE BAG:** For enclosing the strips not in use — 1unit
- **PACKAGE INSERT** — 1copy
- **PLASTIC FILM** — 3sheets

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 450/630nm, microwell aspiration/wash system.

SPECIMENS COLLECTION, TRANSPORTATION AND STORAGE

1. **Specimens Collection**: No special patient's preparation is required. Collect the specimens in accordance with the standard laboratory practice. Either fresh serum or plasma specimens can be used with this assay. 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. Serum specimens should be allowed to clot naturally and completely, the serum 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.
3. Plasma specimens collected into EDTA, sodium citrate or heparin can be tested, the plasma should be spun down.
4. ***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. specimens with visible microbial contamination should never be used.
5. AiD™ anti-HIV 1+2 ELISA is intended ONLY for testing of individual serum or plasma specimens. Do not use the assay for testing of cadaver specimens, saliva, urine or other body fluids, or pooled (mixed) blood.
6. **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, specimens should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical specimens and ethological agents.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8°C, do not freeze. To assure maximum performance of AiD™ anti-HIV 1+2 ELISA, during storage, protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

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 volume of specimen 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 specimens/reagents dispensing. Use different disposal pipette tips for each specimen and reagent 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.
15. **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 HBsAg and antibodies to HIV 1/2, HCV, TP. 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.
16. Never eat, drink, smoke, or apply cosmetics in the assay laboratory. Never pipette solutions by mouth.
17. Chemical should be handled and disposed of only in accordance with the current GLP (Good Laboratory Practices) and the local or national regulations.
18. The pipette tips, vials, strips and specimens 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.
19. 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.
20. 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.
21. 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 specimen 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 Wantai technical support for further assistance.

ProClin™ 300
S phrases:
S26-28-36/37/39-45-60-61
R phrases: 43

Do not eat and drink at the laboratory

Ware protective clothing
Ware eye protection

Biohazard
Danger

PROCEDURE

Reagents 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**.

Step1 **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 specimen 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 for the test.

Step2 **Adding specimens:** Add **100µl** of Positive control, Negative control, and specimens into their respective wells except the Blank. **Note: Use a separate disposal pipette tip for each specimens Negative Control, Positive Control to avoid cross-contamination.**

Step3 **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37°C**.

Step4 **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Wash Buffer (350-400µl/well). 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.

Step5 **Adding HRP-Conjugate:** Add **100µl** of HRP-Conjugate into each well except the Blank.

Step6 **Incubating:** Cover the plate with the plate cover and incubate for **30 minutes at 37°C**.

Step7 **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted Washing buffer (350-400µl/well). 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 to remove any remainders.

Step8 **Coloring:** Add **50µl** of Chromogen A and **50µl** of Chromogen B solutions into each well including the Blank. 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 anti-HIV 1/2 positive specimens wells.

Step9 **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 anti-HIV 1/2 positive specimen wells.

Step10 **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 **10 minutes** after stopping the reaction).

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 specimens 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.

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 specimens 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).

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 specimen 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 450/630nm 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 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**	
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

INTERPRETATIONS OF THE RESULTS

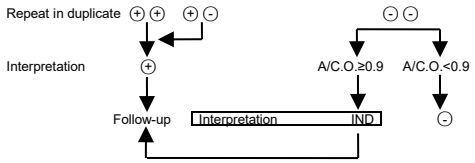
Negative Results (A / C.O. < 1): specimens giving absorbance less than the Cut-off value are non-reactive for this assay, which indicates that no anti-HIV 1/2 antibodies have been detected with AiD™ anti-HIV 1+2 ELISA, therefore the patient is probably not infected with HIV 1/2 and the blood unit do not contain antibodies to HIV 1/2 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 anti-HIV 1/2 antibodies have been detected using AiD™ anti-HIV 1+2 ELISA. All initially reactive specimens should be retested in duplicate using AiD™ anti-HIV 1+2 ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered reactive for antibodies to HIV 1/2 with AiD™ anti-HIV 1+2 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 duplicate is required to confirm the initial results.

Follow-up, confirmation and supplementary testing of any repeatedly reactive specimen 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 INITIALY REACTIVE OR BORDERLINE SPECIMENS



IND = non interpretable

- If, after retesting of the initially reactive specimens, both wells are negative results (A/C.O.<0.9), these specimens 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, etc. For more information regarding Wantai ELISA Troubleshooting, please refer to Wantai's "ELISAs and Troubleshooting Guide".
- If after retesting in duplicate, 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 reactive for antibodies to HIV 1/2 and therefore the patient is probably infected with HIV 1/2 and the blood unit must be discarded.
- After retesting in duplicate, specimens with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone specimens, or uninterpretable for the time of testing.

PERFORMANCE CHARACTERISTICS

Evaluation study carried in Alkmaar, the Netherlands, between April and November 2005, demonstrated the following performance characteristics of AiD™ anti-HIV 1+2 ELISA: The diagnostic specificity of the kit was 99.85% as determined on all negative specimens (5471) that were investigated. When examined on the unselected donors only (random and first time donors), the specificity was 99.92% (95% CI 99.84-100%).

AiD™ anti-HIV 1+2 ELISA test results on unselected donors:

Panel	Number tested	Positive (A/C.O. ≥ 1)		Negative (A/C.O. < 1)	
		Number	%	Number	%
Radom serum donor	2654	2	0.08	2652	99.92
Random plasma donor	1400	1	0.07	1399	99.93
First time donor	989	1	0.10	988	99.90
Total	5043	4	0.08	5039	99.92

All panels of HIV-1, HIV-1 subtype O and HIV-2 confirmed antibody positive specimens that were used in this study were also tested reactive with AiD™ anti-HIV 1+2 ELISA which resulted in diagnostic sensitivity of 100%.

A total of 32 seroconversion panels, which represent 210 specimens tested. 13 specimens not classified from PRB918 and PRB917 because there are not data of Antigen or RNA determination required for the classification. 41 specimens classified as negative. RNA and or Antigen negative. 61 specimens classified as early-seroconversion. 95 specimens classified as seroconversion.

25 positive fresh serum specimens tested in INSTITUTE FOR TROPICAL MEDICINE, BELGIUM have been tested with AiD™ anti-HIV 1+2 ELISA. All 25 positive fresh serum specimens have been positive with AiD™ anti-HIV 1+2 ELISA.

The testing results also show that AiD™ anti-HIV 1+2 ELISA is a state-of-the-art compare to most of the currently available on the market CE-marked tests.

The analytical sensitivity was evaluated on PeliCheck anti-HIV panels. The analytical sensitivity of AiD™ anti-HIV 1+2 ELISA on the PeliCheck anti-HIV standard dilutions was comparable to other anti-HIV assays.

Analytical specificity: AiD™ anti-HIV 1+2 ELISA test results on specimens from hospitalized patients and specimens containing potentially cross-reacting blood-specimens.

Type of specimens	Number tested	Positive (A/C.O. ≥ 1)		Negative (A/C.O. < 1)	
		Number	%	Number	%
Mononucleosis	296	4	1.35	292	98.65
Pregnant woman	101	0	0	101	100
RF+	17	0	0	17	100
Anti-TPO	5	0	0	5	100
Anti-smooth muscle	5	0	0	5	100
Elevated IgG levels	4	0	0	4	100
Total	428	4	0.93	424	99.07

In a separate study, the following specificity results were obtained:

- Possible high dose hook effect is eliminated due to the implementation of two-step procedure.
- Frozen positive/negative specimens have been tested to check for interferences due to collection and storage. The performance characteristics of AiD™ anti-HIV 1+2 ELISA were not affected for at least 3 freeze/thaw cycles.
- specimens from patents infected with hepatitis A, B, C as well as specimens from patients infected with Treponema pallidum were tested with no cross-reactive reactions observed.

Accuracy: The below tables represent the results of analytical sensitivity and reproducibility of AiD™ anti-HIV 1+2 ELISA as controlled with PeliSpy Multi-Marker run control and with Wantai QC specimen tested in every plate - the 1:2048 dilution of the anti-HIV standard in this PeliSpy specimens was consistently detected in all plates. Wantai's QC specimen was always detected in all plates.

PeliSpy Multi-Marker results:

Dilution	n	Average	Percentiles		Measured	
			5th	95th	Min.	Max.
1:2048	80	3.08	1.76	4.39	1.70	4.96

Wantai's QC specimen results:

n	Average	Percentiles		Measured	
		5th	95th	Min.	Max.
160	7.71	5.32	10.10	4.37	10.89

LIMITATIONS

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antibodies may be undetectable during the early stage of the disease and in some immunosuppressed individuals. Therefore, negative results obtained with AiD™ anti-HIV 1+2 ELISA are only indication that the specimen does not contain detectable level of anti-HIV 1/2 antibodies 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 specimen, the assay results are negative, such specimen 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, etc. For more information regarding Wantai ELISA Troubleshooting, please refer to Wantai's "ELISAs and Troubleshooting Guide", or contact Wantai 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) plasma. AiD™ anti-HIV 1+2 ELISA has been evaluated only with individual serum or plasma specimens.
7. AiD™ anti-HIV 1+2 ELISA is a qualitative assay and the results cannot be used to measure antibody concentration. This assay cannot 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 acquired immunodeficiency syndrome (AIDS), Science, 220: 868-871.
2. Barbe, F.et al., (1994) Early detection of anti bodies 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.

SUMMARY OF THE MAJOR COMPONENTS OF THE KIT:

Use this summary only as a reference and always follow the comprehensive method sheet when performing the assay. Note: the components of individual kits are not lot- interchangeable.

1. Microwell plate	Code 5	one
2. Negative Control	Code 8	1x1ml
3. Positive Control (HIV-1)	Code 7_1	1x1ml
4. Positive Control (HIV-2)	Code 7_2	1x1ml
5. HRP-Conjugate	Code 6	1x12ml
6. Wash Buffer	Code 1	1x50ml
7. Chromogen Solution A	Code 2	1x8ml
8. Chromogen Solution B	Code 3	1x8ml
9. Stop Solution	Code 4	1x8ml

SUMMARY OF THE ASSAY PROCEDURE:

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

Add specimens	100µl
Incubate	30minutes
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 450/630 nm

EXAMPLE SCHEME OF CONTROLS / SPECIMENS DISPENSING:

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	S3										
B	Neg.	…										
C	Neg.	…										
D	Neg.											
E	Pos.-1											
F	Pos.-2											
G	S1											
H	S2											

SYMBOLS:

IVD **In Vitro Diagnostic Medical Device**

+2°C~+8°C Storage Conditions

Use By

LOT **Batch**

Content Sufficient For <n> Tests

Instructions For Use

REF **Catalog Number**

Manufacturer

Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.
No.31 Kexueyuan Road, Changping District, Beijing 102206, China
Tel: +86-10-59528888, Fax: +86-10-89705849
Website: www.ystwt.com
Email: wtexport@ystwt.com

Version: V. 2015-01 [Eng.]
Issuing Date: November 11, 2015
Number of revision: Revision 10