Wantai Hepatitis B Virus Diagnostics

AiD™ HBsAg ELISA

Diagnostic Kit for Hepatitis B Virus Surface Antigen (ELISA)

REF WB-1296 | V. 2022-01 [Eng.] | Σ 96 | 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™ HBsAg ELISA achieved.

INTENDED USE

AiD™ HBsAg ELISA is an enzyme-linked immunosorbent assay (ELISA) for qualitative detection of HBsAg in human serum or plasma. It is intended for screening of blood donors and for diagnosing of patients related to infection with hepatitis B virus.

SUMMARY

Hepatitis B virus (HBV) is an enveloped, double-stranded DNA virus belonging to the Hepadnaviridae family and is recognized as the major cause of blood transmitted hepatitis together with hepatitis C virus (HCV). Infection with HBV induces a spectrum of clinical manifestations ranging from mild, inapparent disease to fulminant hepatitis, severe chronic liver diseases, which in some cases can lead to cirrhosis and carcinoma of the liver. Classification of a hepatitis B infection requires the identification of several serological markers expressed during three phases (incubation, acute and convalescent) of the infection. Now several diagnostic test are used for screening, clinical diagnosis and management of the disease. Hepatitis B surface antigen or HBsAg, previously described as Australia antigen, is the most important protein of the envelope of Hepatitis B Virus. The surface antigen contains the determinant “a”, common to all known viral subtypes and immunologically distinguished in two distinct subgroups (ay and ad). HBV has 10 major serotypes and four HBsAg subtypes have been recognized (adw, ady, ayw, and ayr). HBsAg can be detected 2 to 4 weeks before the ALT levels become abnormal and 3 to 5 weeks before symptoms develop. The serological detection of HBsAg is a powerful method for the diagnosis and prevention of HBV infection and ELISA has become an extensively used analytical system for screening of blood donors and clinical diagnosis of HBV in infected individuals.

PRINCIPLE OF THE TEST

For detection of HBsAg, AiD™ HBsAg ELISA uses antibody “sandwich” ELISA method in which, polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBsAg. Patient’s serum or plasma specimen is added to the microwells. During incubation, the specific immunocomplex formed in case of presence of HBsAg in the specimen, is captured on the solid phase. Then the second antibody conjugated the enzyme horseradish peroxidase (the HRP-Conjugate) directed against a different epitope of HBsAg is added into the wells. During the second incubation step, these HRP-conjugated antibodies will be bound to any anti-HBs-HBsAg complexes previously formed during the first incubation, and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing tetramethyl-benzidine (TMB) and urea peroxide are added to the wells. In presence of the antibody-antigen-antibody(HRP) “sandwich” immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP-conjugate 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 it is proportional to the amount of antigen captured in the wells, and to its amount in the specimen respectively. Wells containing specimens negative for HBsAg remain colorless.

COMPONENTS

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-wellper plate

MICROWELL PLATE: Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. Each well contains monoclonal antibodies reactive to HBsAg (anti-HBs). The microwell strips can be broken to be used separately. Place unused wells or strips in the provided plastic sealable storage bag together with the desiccant and return to 2-8°C. Once opened, stable for 4 weeks at 2-8°C.

CONTROL | -
Code8 (1x1ml per vial)
preserv.0.1% ProClin™ 300

NEGATIVE CONTROL: Yellowish liquid filled in a vial with green screw cap.
Protein-stabilized buffer tested non-reactive for HBsAg.
Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.

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

POSITIVE CONTROL: Red-colored liquid filled in a vial with red screw cap.
HBsAg diluted in protein-stabilized buffer.
Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.

HRP | CON
Code 6 (1x6ml per vial)
preserv.0.1% ProClin™ 300

HRP-CONJUGATE: Red-colored liquid in a white vial with red screw cap.
Horseradish peroxidase-conjugated anti-HBs.
Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.

DIL | SPE
Code 9 (1x5ml per vial)
preserv.0.1% ProClin™ 300

SPECIMEN DILUENT: Green-colored in a vial with blue screw cap.
Buffer solution containing protein.
Ready to use as supplied. Once opened, stable for 4 weeks at 2-8°C.

WASH | BUF | 20X
Code 1 (1x30ml per bottle)
DILUTE BEFORE USE!
detergent Tween-20

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.

CHROM | SOL | A
Code 2 (1x6ml 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 opened, stable for 4 weeks at 2-8°C.

CHROM | SOL | B
Code 3 (1x6ml per vial)

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.

STOP | SOL
Code 4 (1x6ml 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 opened, stable for 4 weeks at 2-8°C.

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

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

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. Specimens with visible microbial contamination should never be used.
3. AiD™ HBsAg 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.
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, 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™ HBsAg ELISA, during storage, protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

TO BE USED ONLY BY 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 specimen as indicated in the procedure steps. Failure to do so, may cause low sensitivity of the assay.
5. Do not touch the exterior bottom 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 long time interruptions of assay steps. 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 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 450/600~650nm.
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 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.
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 specimens 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.

Warning:
H317, H412, P273, P280, P333+P313, P363
ProClin™ 300

Danger:
H360D, P201, P280, P308+P313
N,N-dimethylformamide

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**.

Step 1 **Preparation:** Mark three wells as Negative control **(e.g. B1, C1, D1)**, two wells as Positive control **(e.g. E1, F1)** and one Blank (**e.g. A1**, neither specimens 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.

Step 2 **Adding Diluent:** Add **20µl** of Specimen Diluent into each well except the Blank.

Step 3 **Adding Specimen:** Add **100µl** of Positive control, Negative control, and Specimen into their respective wells except the Blank. **Note: Use a separate disposal pipette tip for each specimen, Negative Control, Positive Control to avoid cross-contamination.** Mix by tapping the plate gently.

Step 4 **Incubating:** Cover the plate with the plate cover and incubate **at 37°C for 60 minutes**.

Step 5 **Adding HRP-Conjugate:** At the end of the incubation, remove and discard the plate cover. Add **50µl** of HRP-Conjugate into each well except the Blank, and mix by tapping the plate gently.

Step 6 **Incubating:** Cover the plate with the plate cover and incubate **at 37°C for 30 minutes**.

Step 7 **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.

Step 8 **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 30 minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and HBsAg positive specimen wells.

Step 9 **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 HBsAg positive specimen wells.

Step 10 **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).

INSTRUCTIONSFORWASHING

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.

QUALITY CONTROL AND CALCULATION OF THE RESULTS

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each specimen absorbance (A)

value to the Cut-off value (C.O.) of the plate. 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 + 0.06
(**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 450nm.
- 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**	
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 + 0.06 = 0.076

INTERPRETATIONS OF THE RESULTS

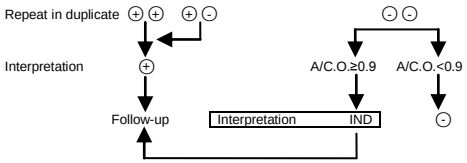
Negative Results (A / C.O. < 1): Specimens giving absorbance less than the Cut-off value are negative for this assay, which indicates that no hepatitis B virus surface antigen has been detected with AiD™ HBsAg ELISA, therefore the patient is probably not infected with HBV and the blood unit do not contain hepatitis B virus surface 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 hepatitis B virus surface antigen has probably been detected using AiD™ HBsAg ELISA. All initially reactive specimens should be retested in duplicate using AiD™ HBsAg ELISA before the final assay results interpretation. Repeatedly reactive specimens can be considered positive for hepatitis B virus surface antigen with AiD™ HBsAg 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 positive specimen with other analytical system (e.g. 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 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. 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 positive for hepatitis B virus surface antigen and therefore the patient is probably infected with HBV 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 specimen, or uninterpretable for the time of testing.

PERFORMANCE CHARACTERISTICS

Evaluation studies carried out in Paul-Ehrlich-Institut (PEI), German Red Cross Institute Baden-Württemberg – Hessen, and three blood banks in China, demonstrated the following performance characteristics of AiD™ HBsAg ELISA.

Specificity: When evaluated on European blood donors (n=5038), the overall diagnostic specificity of the kit was 99.78%.

During multi-center evaluation in China, AiD™ HBsAg ELISA demonstrated specificity of 99.92%.

Laboratory	Number	AiD™ HBsAg ELISA		
		-	+	Specificity
Xiamen blood bank	1958	1955	3	99.85%
Hubei blood bank	2518	2516	2	99.92%
Zhejiang blood bank	6344	6340	4	99.94%
Total	10820	10811	9	99.92%

Sensitivity: AiD™ HBsAg ELISA was evaluated for sensitivity on 22 HBV commercial available HBV seroconversion panels, and on total 403 HBsAg positive including 146 HBsAg HBV genotyped and HBsAg subtyped plasma specimens available at the Paul-Ehrlich-Institut. With respect to seroconversion sensitivity, the results for AiD™ HBsAg ELISA on the 22 HBV seroconversion panels showed a sensitivity level at least equivalent with the range of current CE marked HBsAg screening assays for which PEI holds data.10 additional seroconversion panels were tested in-house. The seroconversion sensitivity was comparable to other CE-marked HBsAg screening test. With respect to diagnostic sensitivity AiD™ HBsAg ELISA detected all positive specimens as positive, including the HBV genotypes A-F or HBsAg subtypes examined.

In conclusion, the overall score of AiD™ HBsAg ELISA for the seroconversion sensitivity was comparable with other CE marked HBsAg test kits for which PEI holds data and all 403 HBsAg positive specimens were reactive giving an overall sensitivity of 100%.

Analytical sensitivity: 0.067IU/ml (NIBSC 00/588)

Analytical specificity: No interference was observed with specimens from patients with high-level of rheumatoid factor, and pregnant woman. Same day and frozen specimens have been tested to check for interferences due to collection and storage. Total of 100 specimens reactive for anti-HBc, anti-HCV and anti-HIV-1 were screened for HBsAg with AiD™ HBsAg ELISA.98 out of 100 specimens were negative for HBsAg.200 blood specimens from patients were also tested with AiD™ HBsAg ELISA.191 out of 200 specimens had negative screening results for HBsAg. 8 out of 9 specimens with initial reactive screening results had repeat reactive test results with AiD™ HBsAg ELISA but hepatitis B virus was not confirmed in all cases.

Detection of mutations: Panel of 108 specimens collected by Xiamen University (Xiamen, China) and sequenced by PCR were tested to demonstrate the performance of AiD™ HBsAg ELISA in detection of HBsAg mutations. The results are given in the table below.

Background		Number	AiD™ HBsAg ELISA
adr (+)	wild type	35	33
	4 mutations	5	4
adw (+)	wild type	37	34
	16 mutations	25	24
ayw (+)	wild type	2	2
	2 mutations	2	2
ayr (+)	2 mutations	2	2
Total		108	101

LIMITATIONS

1. Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
2. Antigens may be undetectable during the early stage of the disease. Therefore, negative results obtained with AiD™ HBsAg ELISA are only indication that the specimen does not contain detectable level of hepatitis B virus surface antigen and any negative result should not be considered as conclusive evidence that the individual is not infected with HBV or the blood unit is not infected with HBV.
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 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™ HBsAg ELISA has been evaluated only with individual serum or plasma specimens.
7. AiD™ HBsAg ELISA is a qualitative assay and the results cannot be used to measure antigen concentration.

REFERENCES

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2. J. Y. Weissman, and S. Krugman. 1987. Yeast-recombinant hepatitis B vaccine. Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. JAMA 257:2612–2616. 143. Stevens, C. E., P. T. Toy, P. E. Taylor, T. Lee, and H. Y. Yip. 1992. Prospects for control of hepatitis B virus infection: implications of childhood vaccination and long term protection. Pediatrics 90(Suppl.):170–173.
3. Hurie, M. B., E. E. Mast, and J. P. Davis. 1992. Horizontal transmission of hepatitis B virus infection to U.S. born children of Hmong refugees. Pediatrics 89:269–273.
4. Szmuness, W., C. E. Stevens, E. J. Harley, E. A. Zang, W. R. Olesko, D. C. Williams, R. Sadovsky, J. M. Morrison, and A. Kellner. 1980. Hepatitis B vaccine: demonstration of efficacy in a controlled trial in a high risk population in the U.S. N. Engl. J. Med. 303:833–841.
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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	Code 7	1x1ml
4. HRP-Conjugate	Code 6	1x6ml
5. Specimen Diluent	Code 9	1x5ml
6. Wash Buffer	Code 1	1x30ml
7. Chromogen Solution A	Code 2	1x6ml
8. Chromogen Solution B	Code 3	1x6ml
9. Stop Solution	Code 4	1x6ml

SUMMARY OF THE ASSAY PROCEDURE:
Use this summary only as a reference and always follow the detailed method sheet when performing the assay.

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

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.											
F	Pos.											
G	S1											
H	S2											

CE MARKING 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

CE **CE Marking – IVDD 98/79/EC**

EC REP **EU Authorized Representative**

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

EC REP **Qarad BV**
Cipalstraat 3, 2440 Geel, Belgium
Email: qarad@qarad.com

CE 2854

Digitally signed by Stoianova Elena
Date: 2025.09.12 22:10:53 EEST
Reason: MoldSign Signature
Location: Moldova

MOLDOVA EUROPEANĂ

Version: V. 2022-01 [Eng.]
Issuing Date: February 9, 2022
Number of revision: Revision 0