

AccuDiag™
HBsAb (Quantitative)
ELISA Kit

REF 1710-12



Test	HBsAb Quantitative
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	Quantitative: Positive, Negative Control
Sample	50 µL serum/plasma
Specificity	>99%
Sensitivity	5mIU/ml
Total Time	~ 75 min
Shelf Life	12 Months from the manufacturing date

INTENDED USE

Diagnostic Automation, Inc. Anti-HBs ELISA (Quantitative) is an enzyme linked immunosorbent assay for in vitro quantitative determination of antibodies to hepatitis B virus surface antigen (anti-HBs) in human serum or plasma for clinical purposes and assessing antibody response levels to HBsAg-vaccine. “Export Use Only”

SUMMARY AND EXPLANATION

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 tests are used for screening, clinical diagnosis and management of the disease.

Hepatitis B surface antigen (HBsAg) is an important viral envelope protein, which appears shortly after infection and is a key serological marker for detection and diagnosis of HBV. Clearance during treatment shows recovery and development of neutralizing antibodies (anti-HBs) occurs in 90% of the patients. Due to the introduction of hepatitis B vaccination programs, the detection of anti-HBs has become important method for monitoring of recipients upon vaccination with synthetic and natural HBsAg. The absence of anti-HBs indicates susceptibility to HBV infection. For this, screening for anti-HBs in high-risk populations is recommended for identifying individuals who may benefit from vaccination.

TEST PRINCIPLE

For detection of anti-HBs, this kit uses antigen “sandwich” ELISA method where polystyrene microwell strips are pre-coated with recombinant HBsAg. Patient’s serum or plasma sample is added to the microwells together with a second HBsAg conjugated to Horseradish Peroxidase (HRP-Conjugate). In case of presence of anti-HBs in the sample, the pre-coated and conjugated antigens will be bound to the two variable domains of the antibody and during incubation, the specific immunocomplex formed is captured on the solid phase. After washing to remove sample and unbound HRP-Conjugates, Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells. In presence of the antigen-antibody-antigen(HRP) “sandwich” complex, 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 is proportional to the amount of antibody captured in the wells, and to the sample respectively.

MATERIALS AND COMPONENTS

Materials provided with the test kits

- Plate:** (1x96wells) 8x12/12x8-well per plate.
Blank microwell strips fixed on white strip holder. The plate is sealed in aluminum pouch with desiccant. Each well contains recombinant HBsAg. 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 open, stable for one month at 2-8°C.
 - Calibration Curve Standards:** (6x0.5ml per vial) (preserv.0.1% ProClin™ 300).
Yellowish liquid filled in a vial with green screw cap. The kit contains the following standards: 0mIU/ml, 10mIU/ml, 20mIU/ml, 40mIU/ml, 80mIU/ml and 160mIU/ml. Anti-HBs diluted in protein-stabilized buffer. Ready to use as supplied. Once open, stable for one month at 2-8°C.
 - HRP-Conjugate:** (1x6.5 ml per vial)
Red-colored liquid in a white vial with red screw cap. Horseradish peroxidase-conjugated HBsAg. Ready to use as supplied. Once open, stable for one month at 2-8°C.
 - Wash Buffer:** (1x30ml per bottle) Dilute before use. Buffer solution containing surfactant.
Colorless liquid filled in a white bottle with white screw cap. The concentrate must be diluted **1 to 20** with distilled/deionized water before use. Once diluted, stable for one week at room temperature, or for two weeks when stored at 2-8°C.
 - Chromogen Solution A:** (1x7ml per vial) Colorless liquid filled in a white vial with green screw cap. Urea peroxide solution. Ready to use as supplied. Once open, stable for a month at 2-8°C.
 - Chromogen Solution B:** (1x7ml per vial) Colorless liquid filled in a black vial with black screw cap. TMB solution (Tetramethyl benzidine). Ready to use as supplied. Once open, stable for one month at 2-8°C.
 - Stop Solution:** (1 x 7 ml per vial) Colorless liquid in a white vial with white screw cap.
Diluted sulfuric acid solution (0.5M H₂SO₄). Ready to use as supplied. Once open, stable for one month at 2-8°C.
- PLASTIC SEALABLE BAG:** For enclosing the strips not in use 1 unit
 - PACKAGE INSERT** 1 copy
 - CARDBOARD PLATE COVER** 2 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± 1.0°C, plate reader, single wavelength 450nm or dual wavelength 450/630nm, micro-well aspiration/wash system.

SPECIMEN COLLECTION AND 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 anti-HBs ELISA (Quantitative) 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 7 days should be stored frozen (-20°C or lower). Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

STORAGE

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 anti-HBs ELISA (Quantitative), during storage, protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS

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.

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 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.

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

20. The stop solution 0.5M H₂SO₄ is an acid. Use it with appropriate care. Wipe up spills immediately and wash with water if come into contact with skin or eyes.

21. Pro-Clin 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.



ASSAY 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**.

- Preparation:** Mark six calibration curve standards wells (e.g. B1-G1; H1-E2) and one Blank (e.g. A1, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test. **Run the standards in duplicates.**
- Adding Sample:** Add 50µl of Calibration curve standards and Specimen into their respective wells except the Blank. **Note: Use a separate disposal pipette tip for each specimen and standard to avoid cross-contamination.**
- Adding HRP-Conjugate:** Add 50µl of HRP-Conjugate into each well except the Blank. Mix by tapping the plate gently.
- Incubating:** Cover the plate with the plate cover and incubate for **60 minutes at 37°C**.
- 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.
- 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 Calibration curve standards wells (except for 0mIU/ml) and in anti-HBs positive sample wells.
- Stopping Reaction:** Using a multichannel pipette or manually, add **50 µl** of Stop Solution into each well and mix gently. The blue color will turn yellow after stopping the reaction.
- 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

- A good washing procedure is essential in order to obtain correct and precise analytical data.
- 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.
- 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.
- 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.
- 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.
- 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.
- The concentrated Wash buffer should be diluted **1 to 20** before use. If less than a whole plate is used, prepare the proportional volume of solution.

RESULTS & QUALITY CONTROL

Each microplate should be considered separately when calculating and interpreting the results of the assay, regardless of the number of plates concurrently processed. If the result reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD from the print report values of specimens and standards. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of specimens and standards.

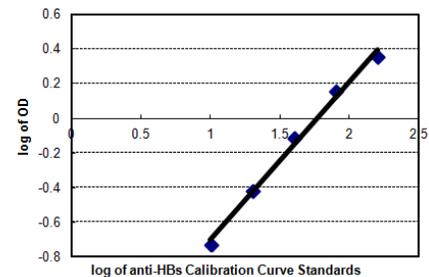
- Record the absorbance (OD) obtained from the print report of the microplate reader.
- Plot the absorbance (log-OD) for each duplicate calibration standard on the Y (logarithmic ordinate) versus the corresponding anti-HBs concentration (log-mIU/ml) on the X (logarithmic abscissa) on double-logarithmic paper (do not average the duplicates of the calibration standards before plotting).
- Draw the standard curve through the plotted points (best-fit).
- To determine the concentration of anti-HBs for an unknown, locate the absorbance (OD) for each unknown on the Y-axis of the graph, find the intersecting point on the standard curve, and read the concentration (log-mIU/ml) from the X-axis of the graph. Calculate the concentration of the unknown in mIU/ml.

Example of a Standard Curve:

For illustration purpose only, the average values are given only: (*0mIU/ml=Negative Sample or Negative Control)

Standards	log mIU/ml	Mean OD	log OD
10mIU/ml	1	0.186	-0.728
20mIU/ml	1.30103	0.380	-0.4202
40mIU/ml	1.60206	0.770	-0.1135
80mIU/ml	1.90309	1.427	0.1544
160mIU/ml	2.20412	2.249	0.3521

Example of anti-HBs Standard Curve



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 OD value of the Blank well, which contains only Chromogen and Stop solution, is < 0.080 at 450 nm.

The OD value of 0mIU/ml standard must be < 0.100 at 450/630nm or at 450nm after blanking.

The OD value of 160mIU/ml standard must be > 1.500 at 450/630nm or at 450nm after blanking.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity (lower detection limit): In the follow-up of vaccinated individuals the value of 10 WHO mIU/ml is the minimum concentration at which the recipient is considered protected. This kit shows sensitivity of 5mIU/ml.

Clinical Sensitivity: The performance characteristics of this assay were evaluated by a panel of samples obtained from 600 individuals receiving HBV vaccines in which the titers of anti-HBs were evaluated in a direct comparison with another commercially available anti-HBs ELISA kit. From this group, 594 individuals showed antibody titer higher than 10mIU/ml, which was confirmed with the reference anti-HBs ELISA kit. In another group of 220 individuals with confirmed hepatitis B vaccination history, 220 of the tested samples showed antibody titer higher than 10mIU/ml. From this study, overall agreement of 100% was obtained between this kit and the reference test in linear regression analysis.

In a panel of 240 samples obtained from early recovery hepatitis B patients (confirmed HBsAg -, anti-HBc + and anti-HBs +), sensitivity of 100% was calculated in comparison with the reference test.

Specificity: > 99% calculated by a panel of samples obtained from 500 healthy individuals with confirmed levels of anti-HBs less than 10mIU/ml.

No cross reactivity observed when testing samples from patients infected with HAV, HCV, HIV, CMV, and TP. No interference from elevated levels of rheumatoid factors up to 2000U/ml. No high dose hook effect up to 150000mIU/ml observed during clinical testing. The kit 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.

Recovery:

HBsAb Added	HBsAb Measured	Recovery
0mIU/ml	-	-
20mIU/ml	19.6mIU/ml	98.00%
76mIU/ml	75.0mIU/ml	98.68%
94mIU/ml	93.7mIU/ml	99.68%
130mIU/ml	149.0mIU/ml	114.62%
190mIU/ml	185.0mIU/ml	97.37%

LIMITATIONS OF PROCEDURE

1. Non- repeatable reactive results may be obtained with any ELISA test due to the general characteristics of this method. Any positive result must be interpreted in conjunction with the patient clinical information and other laboratory results.
2. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are related but not limited to inadequate washing step. For more information regarding Diagnostic Automation, Inc. ELISA Troubleshooting, please refer to Diagnostic Automation, Inc. "ELISAs and Troubleshooting Guide", or contact DAI technical support for further assistance.
3. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
4. The prevalence of the marker will affect the assay's predictive values.
5. In some cases, very strong immunological response after vaccination can be observed due to the vaccine biological characteristics. High concentrations of antibodies beyond the standard curve measurement range (>160mIU/ml) can be diluted and retested. Samples may not show linear properties after dilution as the same way as the materials used for the standards. This phenomenon is frequently observed when samples are tested for antibodies.
6. Samples tested using assay from different manufacturer can give similar quantitative results but some samples can give discrepancies due to the antibodies diversity and the antigenic properties of HBsAg used in the assay.
7. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

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