Reproducibility		Within Run	Between Run
Standards	Test	CV%	CV%
*0mIU/ml	10	14	14
10mIU/ml	10	7.7	8.1
20mIU/ml	10	7	7.5
40mIU/ml	10	6	7.3
80mIU/ml	10	4.5	4.68
160mIU/ml	10	3.9	4.2

***0mIU/ml=Negative Samples or Negative Control.**

LIMITATIONS

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.
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. 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.
5. 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.
6. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.

INDICATIONS OF INSTABILITY OR DETERIORATION OF THE REAGENTS

1. Values of the Positive or Negative controls ,which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones.
2. If after mixing of the Chromogen A and B solutions into the wells, the, the color of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

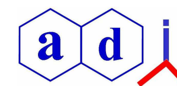
Instruction Manual No. M-4220-AHB

Human Anti-Hepatitis B Surface Antigen (anti-HBsAg) IgG ELISA Kit

Cat. #. 4220-AHB

For **quantitative** determination of anti-HbS in Human Serum in clinical samples or to assess vaccine response

For In-vitro research use only



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For **quantitative** determination of anti-HbS in Human Serum in clinical samples or to assess vaccine response

ELISA kit #. 4220-AHB

Kit Components, 100 strips	Cat #
HBSAg coated ELISA strip plate (12x 8 wells) #4221-AHB	1 plates
0 mIU, 10 mIU, 20 mIU, 40 mIU, 80mIU, 160mIU/ml standards of human anti-HBsAg IgG in protein stabilized buffer; # 4222A-F-AHB	6 x 500 ul vials.
HRP conjugate Reagent (red cap), contains HBSAg-HRP in a protein stabilized buffer, 6.5 ml; #4223-AHB	1 bottle
Stock Wash buffer (20X) white cap; 30 ml; dilute 1:20 with distilled water #4220WB	1 bottle
Chromogen Solution A (green cap); 7 ml #4220-SA	1 bottle
Chromogen Solution B (black cap); 7 ml #4220-SB	1 bottle
Stop Solution B (white vial with white cap); 7 ml #4220-SS	1 bottle
Plastic sealable bag **plate cover**	1 1

INTRODUCTION

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 disease, 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.

This anti-HBs ELISA kit is an enzyme linked immunosorbent assay for in vitro quantitative detection 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.

Quality control range:

The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450nm.
2. The OD value of 0mIU/ml standard must be less than 0.100 at 450/630nm or at 450nm after blanking.
3. The OD value of the 160mIU/ml standard must be higher than 1.500 at 450/630nm or at 450nm after blanking.

TEST PERFORMANCE AND EXPECTED RESULTS

Analytical Endpoint Sensitivity: (lower detection limit): In the follow-up of vaccinated individuals the value of 20 WHO mIU/ml is the minimum concentration at witch the recipient is considered protected. This anti-HBs ELISA 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 titers higher than 10mIU, 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. From this study, overall agreement of 100% was obtained between this anti-HBs ELISA 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 10 mIU/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 mIU/ml	*HBsAb measured* mIU/ml	**Recovery %**
0mIU/ml	-	-
20mIU/ml	19.6mIU/ml	98
76mIU/ml	75.0mIU/ml	98.68
94mIU/ml	93.7mIU/ml	99.68
130mIU/ml	149mIU/ml	114
190mIU/ml	185mIU/ml	97.36

INTERPRETATION OF RESULTS AND QUALITY CONTROL

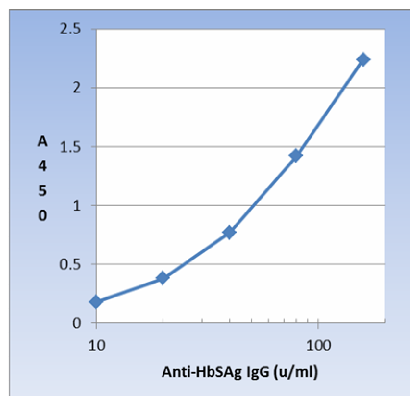
If the results reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report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

1. Record the A450 (OD) obtained from the print report of the microplate reader.
2. 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).
3. Draw the standard curve through the plotted points (best-fit).
4. 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-ng/ml) from the X-axis of the graph. Calculate the concentration of the unknown in ng/ml.

Example of a Standard Curve:

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

Standards	*Mean OD*
10mIU/ml	0.186
20mIU/ml	0.380
40mIU/ml	0.770
80mIU/ml	1.427
160mIU/ml	2.249



PRINCIPLE OF THE TEST

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 EQUIPMENT REQUIRED

Freshly distilled or deionized water; Disposable gloves and timer; Appropriate waste containers for potentially contaminated materials.; Disposable V-shaped troughs; Dispensing system and/or pipette (single or multichannel), disposable pipette tips; Absorbent tissue or clean towel; Dry incubator or water bath, 37±0.5; Microshaker for dissolving and mixing conjugate with samples.
Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm; Microwell aspiration/wash system

PRECAUTIONS AND SAFETY INSTRUCTIONS

This kit is intended FOR IN VITRO USE ONLY
The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. CAUTION - CRITICAL STEP: Allow the reagents and samples to stabilize at room temperature(18-30℃) before use. Shake reagent gently before, and return to 2-8℃ 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 microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.

8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. All specimens from human origin should be considered as potentially infectious.
11. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV ½, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
12. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
13. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121R or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

Sample Collection: Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.

Transportation and Storage: Store samples at 2-8℃. Samples not required for assaying within 3 days should be stored frozen (-20℃ or lower).Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

STORAGE AND STABILITY

Store at 4oC. if unopened, are stable at 2-8°C until the expiration date printed on the label (usually 1 year from the date of manufacture).

ASSAY PROCEDURE

Step1 **Reagents preparation:** Allow the reagents and samples to reach room temperature (18-30℃) for at least 15-30minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37℃ until crystals dissolve. Dilute the stock wash Buffer **1 to 20** with distilled or deionized water. Use only clean vessels to dilute the buffer.

Step2 **Numbering Wells:** Set the strips needed in strip-holder and number sufficient number of wells including 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.

Step3 **Adding Sample:** Add **50µl** of Calibration curve standards and **50µl** specimen into their respective wells. **Note: Use a separate disposal pipette tip for each specimen as to avoid cross-contamination.**

Step4 **Adding HRP-Conjugate:** Add **50µl** of HRP-Conjugate Reagent into each well except into the Blank and mix gently. **Never add HRP-Conjugate to the Blank well.**

Step5 **Incubating:** Cover the plate with the plate cover and incubate for **60minutes at 37°C**. It is recommended to use thermostat-controlled water tank as to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

Step6 **Washing:** At the end of the incubation, remove and discard the plate sealer. Wash each well **5times** with diluted Wash buffer. Each time, allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the plate onto blotting paper or clean towel, and tap it to remove any remainders.

Step7 **Coloring:** Dispense **50µl** of Chromogen A and **50µl** Chromogen B solution into each well including the **Blank** and mix by tapping the plate gently. Alternatively, Soln A and B can be mixed (1:1 ratio) and then add 100 ul in a single step. Incubate the plate at **37℃ for 15minutes avoiding light**. The enzymatic reaction between the Chromogen A/B solutions and the HRP-Conjugate produces blue color in Calibration curve standards wells (except for 0mIU/ml) and in anti-HBs positive sample wells.

Step8 **Stopping Reaction:** Using a multichannel pipette or manually add **50µl** Stop Solution into each well and mix gently. The blue color will turn yellow after stopping the reaction.

Step9 **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 results (**Note:** read the absorbance within **10minutes** after stopping the reaction) .