Prestige Diagnostics
Clinical Diagnostics & Point of Care Products

Anti HBs Quant (HBsAb) Elisa

CAT NO	DESCRIPTION	PACK SIZE
EIAHBS1	Anti HBs Elisa	96 Tests

Intended Use:

Anti HBs Elisa (Quantitative) is an enzyme linked immunosorbent assay for in vitro quantitative determination of antibodies to Hepatitis B virus surface antigen in human serum or plasma for clinical purposes and assessing antibody response levels to HBsAg vaccine. This reagent is for In vitro Diagnostic use only.

Summary and Principle:

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.

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.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate)	Each microwell is coated with recombinant HBsAg. The microwells can be broken and used separately. Place unused wells or strips in the provided plastic sealable bag together with the desiccant and store at 2-8°C. Once open the wells are stable for 1 month at 2-8°C.
Standards	6x0.5ml	Calibration curve standards: Yellowish liquid filled in a vial with green screw cap. The kit contains standards: 0 mIU/ml, 10 mIU/ml, 20 mIU/ml, 40 mIU/ml, 80 mIU/ml and 160 mIU/lm. Ready to use. Once open stable for 1 month at 2-8°C.
HRP-Conjugate	1x6.5ml	Red coloured vial in a white vial with screw cap. Horseradish peroxidise conjugated HBsAg. Once open, stable for one month at 2-8°C.
Wash Buffer (20X)	1x30ml	PBS at pH 7.4. 20X concentrate. Once open, stable for one month at 2-8°C. The concentrate must be diluted 1 to 20 with distilled water before use. Once diluted, stable for one week at room temperature, or two weeks when stored at 2-8°C.
Chromogen A	1x7ml	Urea peroxide solution. Ready to use. Once open, stable for one month at 2-8°C.
Chromogen B	1x7ml	TMB Solution. Ready to use. Once open, stable for one month at 2-8°C.
Stop Solution	1x7ml	Diluted Sulfuric acid solution (0.5M) Ready to use. Once open, stable for 1 month at 2-8°C.

Plastic Sealable bag, IFU and Cardboard plate covers.

Materials provided but not required:

Distilled water or deionized water, disposable gloves and timer, appropriate waste containers for potentially contaminated materials, dispensing systems, disposable pipette tips, absorbent tissue or clean towel, dry bath incubator or water bath, plate reader, single wavelength 450nm or dual wavelength 450/630nm and microwell aspiration systems.

Specimen Collection:

1. No special patient preparation is required. Collect the specimen in accordance with normal laboratory practice. Either fresh serum or plasma specimens can be used with this assay. Blood collected by venepuncture 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 specimens should be removed by centrifugation at 3000 RPM 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 haemolytic specimens should not be used as they 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. The AMS UK Anti HBs Elisa assay is used only for testing individual serum or plasma samples. Do not use for testing 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 at -20°C or lower. Multiple free thaw cycles should be avoided. For shipment, samples should be packaged and labelled in accordance with the existing local and international regulations for transportation of clinical samples and ethological agents.

Storage and Stability:

The contents of the kit will remain stable up to expiry date when stored at 2-8°C. Do not freeze. Keep all components tightly capped and without any contamination.

Precautions and Safety:

The Elisa assays are time and temperature sensitive. To avoid incorrect results, strictly follow the test procedure 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 121oC 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. MSDS available upon request.**
19. **Some reagents may cause toxicity, irritation, burns or have carcinogenic effects as raw materials. Contact with skin and the mucosa should be avoided but not limited to the following reagents: stop solution, chromogen reagents and the wash buffer.**
20. **The stop solution contains sulfuric acid. Use it with appropriate care. Wipe up spills, immediately and wash with water if comes into contact with the skin or the eyes.**
21. **Proclin 300 is used as preservative and can cause sensation of the skin. Wipe up spills immediately or wash with water if comes into contact with skin or eyes.**
22. **INDICATIONS OF INSTABILITY OR DETERIORATION OF THE REAGENTS: The values of positive and 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 cases, the results should be considered as invalid and the samples must be retested. In case of consistently erroneous results and proven deterioration or instability of the reagents, immediately discard the reagents in use and use a new kit. Contact the local AMS UK representative.**

Procedure:

Reagent preparation:

Allow the reagents to reach room temperature (18-30°C). Check the wash buffer concentration for the presence of salt crystals. If crystals have formed, re-solubilize 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 clean vessels to dilute the buffer. All other reagents are ready to use as supplied.

STEP 1

Preparation: Mark six calibration curve standard wells (B1-G1; H1-E2) and one blank (A1, neither samples nor HRP conjugate should be added into the blank well). IF the results will be determined 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.

STEP 2

Addition of the sample: Add 50ul of Calibration curve standards and specimen into their respective wells except the blank. Note: Use a separate disposable tip for each specimen and standard to avoid cross-contamination.

STEP 3

Addition of HRP Conjugate: Add 50ul of HRP Conjugate into all wells except the Blank well.

STEP 4

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

STEP 5

Washing: At the end of the incubation period, 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 a blotting paper or a clean towel and tap it to remove any residual buffer.

STEP 6

Addition of the chromogens: Add 50ul of Chromogen A and 50ul of Chromogen B into each well including the blank. Incubate the plate at 37°C for 15 minutes avoiding light.

STEP 7

Stopping the Reaction: Add 50ul of the Stop solution into each well and mix gently.

STEP 8

Measurement: 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: Absorbances must be read within 10 minutes of adding the stop solution).

Instructions for Washing:

- To remove any effect washing on false positive reactions, a 5 automatic wash cycle is required with 350-400ul of diluted wash buffer used per well per wash. This helps in avoiding false positive reactions and a high background.
- To avoid cross-contamination 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 350-400ul/well and aspirating the liquid 5 times. If poor results are observed with high background, increase washing cycles to soak time per well.
- Treat the liquid aspirated after the reaction from the wells with Sodium hypochlorite (at a concentration of 2.5%) for 24 hours before they are disposed off in the appropriate way.
- 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.

Calculation of results:

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.

1. Record the absorbance obtained from the microplate reader.

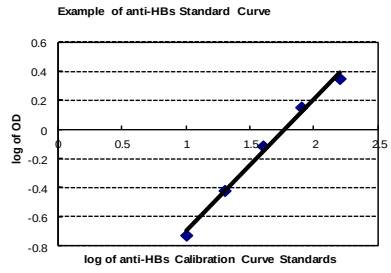
2. Plot the absorbance (log-OD) for each duplicate calibration standard on the Y (logarithmic ordinate) versus the corresponding anti-HBs concentration (log-mIU/ml) on the X (logarithmic abscissa) on double-logarithmic paper (do not average the duplicates of the calibration standards before plotting).

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-mIU/ml) from the X-axis of the graph. Calculate the concentration of the unknown in mIU/ml.

STANDARD CURVE EXAMPLE:

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



Test results are valid if the QC criteria are fulfilled. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar or identical with the patient sample tested.

- The OD value of the Blank well, which contains only Chromogen and stop solution is <0.080 at 450nm.
- The OD value of 0 mIU/ml standard must be <0.100 at 450/630nm or at 450nm after blanking.
- The OD value of 160 mIU/ml standard must be >1.500 at 450/630nm or at 450nm after blanking.

Performance Characteristics:

The kit was standardized against reference standards.

Analytical Sensitivity (lower detection limit): In the follow-up of vaccinated individuals the value of 20 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
0 mIU/ml	-	-
20 mIU/ml	19.6 mIU/ml	98.00%
76 mIU/ml	75.0 mIU/ml	96.68%
94 mIU/ml	93.7 mIU/ml	99.68%
130 mIU/ml	149.0 mIU/ml	114.61%
190 mIU/ml	185.0 mIU/ml	97.36%

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. 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.

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.

References:

1. Lewis, T., et al. (1972). A Comparison of the frequency of hepatitis-B Antigen and antibody in hospital and non-hospital personnel. New Engl. J. Med., 289, 647
2. Hadler, S.C., et al. (1986). Long-term Immunogenicity and Efficacy of Hepatitis B vaccine in homosexual men. New Engl. J. Med. 315, 209
3. Jilg, W., et al. (1989). Vaccination against Hepatitis B: Comparison of three different vaccination schedules. J. Infect. Dis., 160, 766
4. Jilg, W., et al. (1990). Hepatitis B–vaccination strategy for booster doses in high-risk population groups. Progress in Hepatitis B Immunisation. P. Coursaget, M.J. Tong eds., Colloque INSERM. 194, 419
5. Engvall E. and Perlmann P. J.Immunochemistry, 8, 871-874, 1971
6. Engvall E. and Perlmann P. J.Immunol. 109, 129-135, 1971

REF	Catalog number		Temperature limitation
	Consult instructions for use	LOT	Batch code
IVD	*In vitro* diagnostic medical device		Use by
	Manufacturer		

Validation: