HBsAg ELISA

CAT NO	DESCRIPTION	PACK SIZE
EIAHBG1	HBsAg ELISA (Hepatitis B Surface Antigen)	96 Tests
EIAHBG2	HBsAg ELISA (Hepatitis B Surface Antigen)	480 Tests

Intended Use:

HBsAg ELISA is an enzyme linked immunosorbent assay (ELISA) for the qualitative detection of Hepatitis B surface antigen in human serum and plasma. It is intended for screening of blood donors and for diagnosing of patients suspected of infection with hepatitis B virus. 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 symptoms ranging from mild, unapparent disease to full 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 of the infection (incubation, acute and convalescent). Several diagnostic tests are now used for screening, clinical diagnosis and management of the disease. Hepatitis B surface antigen (HBsAg) 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.

For detection of HBsAg, the HBsAg ELISA uses a double antibody sandwich ELISA method in which microwells are pre-coated with monoclonal antibodies against Hepatitis B surface antigen. Samples are added to the microwells along with another anti-HBs antibody, this one conjugated to the enzyme horseradish peroxidase (HRP), and directed against a different epitope of HBsAg. During incubation, if HBsAg is present in the sample, it will bind with and by captured by both the surface and conjugate antibodies forming labelled immunocomplexes which are bound to the plate surface. Unbound sample proteins and conjugate are removed by a wash step. Chromogen solutions containing tetramethyl-benzidine (TMB) and urea peroxide are added to the wells where the HRP on the immunocomplexes catalyses the hydrolysis of the chromogens to form a blue coloured product. The blue colour turns yellow after stopping the reaction with sulphuric acid. The colour intensity can be measured spectrophotometrically and is proportional to the amount of antigen in the sample. Wells containing samples negative for HBsAg remain colourless.

Reagent Composition:

COMPONENT	SIZE	DESCRIPTION
Microwell Plate	1x96 wells (12x8 well plate) 5x96 (480T)	Each microwell is coated with antibodies to 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.
Negative Control	1x1ml 3x1ml (480T)	Protein stabilized buffer tested non-reactive for HBsAg. Yellow in colour. Ready to use. Once open stable for 1 month at 2 - 8°C.
Positive Control	1x1ml 3x1ml (480T)	Protein stabilized buffer with dilution of HBsAg. Red coloured solution. Once open, stable for 1 month at 2 - 8°C.
HRP-Conjugate	1x7ml 5x7ml (480T)	Red coloured liquid. HRP conjugated anti-HBs antibody. Once open, stable for one month at 2 - 8°C.
Wash Buffer (20X)	1x30ml 2x100ml (480T)	PBS at pH 7.4, 20X concentrate. The concentrate must be diluted 1/20 with distilled water before use. Once diluted it is stable at room temperature for a week or two weeks at 2 - 8°C.
Chromogen A	1x8ml 1x60ml (480T)	Urea peroxide solution. Ready to use. Once open, stable for one month at 2 - 8°C.
Chromogen B	1x8ml 1x60ml (480T)	TMB Solution. Ready to use. Once open, stable for one month at 2 - 8°C.
Stop Solution	1x8ml 1x60ml (480T)	0.5M Sulphuric acid solution. Ready to use. Once open, stable for 1 month at 2 - 8°C.

Plastic Sealable bag, IFU and Cardboard plate covers.

Materials required but not provided:

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:

Collect serum and EDTA, sodium citrate or heparin plasma by separation after standard venipuncture technique. For serum, the blood must be allowed to clot fully but be separated as soon as possible to avoid haemolysis of the RBC. Any visible particulate matter in the serum or plasma should be removed by centrifugation. Do not heat inactivate samples.

Samples will be stable for analysis up to 3 days at 2 - 8°C or stored at -20°C.

Do not use samples with high content of haemoglobin, bilirubin or triglyceride as these substances can interfere in the ELISA.

The HCV ELISA assay is used only for testing serum or plasma samples. Do not use for testing cadaver samples, saliva, urine or other body fluids or pooled blood.

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, follow the test procedure exactly as specified.

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 expiry indicated on the kit box and of the same lot. Never use reagents beyond their expiry date stated on labels.**
3. **IMPORTANT: Allow the reagents and samples to reach room temperature (15 - 30°C) before use. Shake reagent gently before use. Return at 2 - 8°C immediately after use.**
4. **Do not touch the bottom of the plate; 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.**
5. **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.**
6. **Make sure timing of reagent additions are the same for all wells and for each procedure step.**
7. **Make sure the temperature inside the incubator is 37°C.**
8. **Use different pipette tip for each sample and reagent in order to avoid cross-contamination. Only touch the pipette tip against the side of the well, never the bottom of the well.**
9. **The enzyme activity of the HRP-conjugate is affected by dust and strong chemicals including sodium hypochlorite, acids and alkalis. Do not let these substances contaminate the Conjugate reagent.**
10. **If using fully automated equipment do not cover the plate with a plate cover during incubation. The tapping out of well contents after washing, can also be omitted.**
11. **All samples from human origin should be considered as potentially infectious. Strict adherence to Good Laboratory Practice regulations will ensure personal safety**
12. **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 samples or reagents are completely absent. Therefore, handle reagents and samples 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.**
13. **Pipette tips, vials, well strips and sample 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. Reagents must be disposed of only in accordance with local or national regulations**
14. **Some reagent components may cause toxicity, irritation, burns or have carcinogenic effects as raw materials. Avoid contact of all reagents with skin and the mucosa but especially the Stop Solution, Substrate reagents and the Wash Buffer.**
15. **The Stop Solution contains sulphuric acid. Use it with appropriate care. Wipe up spills, immediately and wash with water if comes into contact with the skin or the eyes.**
16. **Proclin 300 is used as preservative and can cause a reaction on the skin. Wipe up spills immediately or wash with water if comes into contact with skin or eyes.**
17. **Indicators of deterioration of the reagents: The values of Positive and Negative controls fall out of the quality control range may indicate reagent instability or operator or equipment error. Where quality controls are out of range the results should be considered invalid and the samples must be retested. In case of consistently erroneous results and proven deterioration of the reagents, immediately discard the reagents in use and use a new kit. Contact the local Prestige Diagnostics representative.**

Procedure:

Reagent preparation:

Allow the reagents to reach room temperature (15 - 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), for example by adding 30 ml Wash Buffer concentrate to 570 ml of distilled or deionized water. Alternately add concentrate to distilled water in the ratio 1/20. All other reagents are ready to use as supplied.

STEP 1

Preparation: Reserve 3 wells for Negative Control, 2 wells for Positive Control and one Blank (e.g. A1 – taking care that neither HRP conjugate nor any samples are added to the blank well). Note: If results are read using a plate reader having dual wavelength (450 / 600-650nm) then a Blank well need not be used. Assign samples to wells.

STEP 2

Addition of samples: Add 50 µl of Positive control, Negative Control and samples to their assigned wells except the Blank.

STEP 3

Addition of conjugate: Add 50 µl of HRP Conjugate to each well except the Blank well and mix by tapping the plate gently.

STEP 4

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

STEP 4

Washing: At the end of the incubation, remove the plate cover and discard the well contents by decantation or aspiration. Add 350 µl of diluted wash solution to all wells and soak for one minute before discarding the buffer. Repeat 4 more times for a total of 5 washes. Use of an automated microplate strip washer is recommended. At the end of washing, invert the plate and tap out any residual wash solution onto absorbent paper.

STEP 5

Addition of the chromogens: Add 50 µl of Chromogen A and 50 µl of Chromogen B to each well including the blank. Incubate the plate at 37°C for 15 minutes. Ensure this incubation is carried out in the dark.

STEP 6

Stopping the Reaction: Add 50 µl of Stop Solution to each well and mix gently until the blue colour changes completely to yellow.

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 determine the results. (Note: Absorbances must be read within 10 minutes of adding the Stop Solution).

Instructions for Washing:

To remove the potential for poor washing to cause false positive results and a high background, a 5 step automatic wash cycle is required with 350-400ul of diluted wash buffer used per well per wash.
Ensure the microplate washer is adequately maintained. Ensure the liquid dispensing channels are not blocked or contaminated and that correct volume of wash buffer is dispensed each time into the wells.
If plates have to be washed manually, a process of 5 washing cycles, dispensing 350-400ul/well, allowing 60 second soak and aspirating the liquid 5 times is recommended. If poor results are observed with high background, increase the number of washing cycles and soak time per well.
Capture all liquid waste aspirated and treat with 2.5% sodium hypochlorite for 24 hours before being disposed of in the appropriate way.

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 run. The results are calculated by relating each sample absorbance (Abs) with the cut off value (CO) 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 Abs from the absorbances of the samples and the controls. If the results are taken from a dual filter plate reader, do not subtract the Blank well Abs value from the sample and control absorbances, just use the values obtained.

Calculation:

Cut off value (CO) = Nc x 2.1
(Nc = the mean absorbance value for 3 negative controls).
Important: If the mean Abs value of the Negative Controls is less than 0.050 then use 0.05 as the Nc value.

Validation:

The assay results are only valid if the following Quality Control criteria are met:
Blank well: the absorbance must be <0.080 at 450nm.
Positive Control: the absorbance must be ≥ 0.800 at 450/630nm or at 450nm after blanking.
Negative Control: the absorbance must be <0.100 at 450/630nm or at 450nm after blanking.
If one of the Negative Control absorbances does not match the above criteria, this value should be ignored and a mean value should be calculated using the other two values. If more than one Negative Control absorbance does not meet the criteria, the test is invalid and the whole run repeated.

Example:

Blank Value	A1: 0.022	450nm (blanking is required only when reading with a single filter)	
Negative control	0.019	0.018	0.021
Positive control	2.381	2.407	

Calculation of Nc: ((0.019 + 0.018 + 0.021)/3) = 0.019
The calculated Nc is less than 0.05, so use 0.05 as the Nc value
Calculation of the cut off: 0.05 x 2.1 = 0.105

Interpretation of the results:

Negative Results: (Abs /CO <1) Samples giving absorbance less than that of the Cut off value are negative for this assay. This indicates that the sample is non-reactive for HBsAg and the patient is probably not infected with HBV and the blood unit does not contain HBsAg and could be transfused in case other infectious diseases markers are also absent.
Positive Results: (Abs /CO >/= 1) Samples giving an absorbance equal to or greater than the cut off values are considered initially reactive, indicating that HBsAg has been probably detected. All initially reactive samples should be re-tested with this kit before final interpretation. Repeatedly reactive samples can be considered positive for HBsAg.
Borderline: (Abs /CO = 0.9 – 1.1) Samples with absorbance to cut off ratio between 0.9 to 1.1 are considered borderline and retesting of these samples in duplicates is required to confirm the initial results.

Follow up, confirmation and supplementary testing of any positive sample with other analytical systems such as PCR is required. Clinical diagnosis should not be established using a single result.
If, after re-testing of initially reactive samples, both wells show negative results (Abs/CO < 0.9), these samples should be considered negative and the original result must be classified as false positive. False positive results may occur due to several reasons, often associated with, but not limited to inadequate washing step.
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 samples should be regarded as positive for HBsAg and the blood unit must be discarded.
After retesting in duplicate, samples with values close to the Cut-off value should be interpreted with caution and considered as borderline, or uninterpretable at the time of sample taking.

Performance Characteristics:

Clinical Specificity: The clinical specificity of this assay was determined using a panel of samples from 2500 healthy donors and 300 undiagnosed hospitalized patients. Results are as follows:

No. samples	Negative	Positive	Confirmed +ve	False Pos	Specificity
2500	2444	56	53	3	99.87%
300	273	27	26	1	99.63%

Clinical Sensitivity: Clinical sensitivity was assessed using samples from 670 well characterized Hepatitis B positive patients confirmed by testing with a predicate ELISA

Type	No.	Neg	Pos	Confirmed +ve	False -ve	Sensitivity
Acute	200	0	200	200	0	100%
Chronic	400	1	399	400	1	99.75%
Recovery	70	65	5	5	0	100%

Analytical Endpoint Sensitivity: The sensitivity of this assay has been calculated by means of reference standards and was found to be at 0.5 ng/ml (adr) and 0.5 ng/ml (adw, ay).
Cross Reactivity: No cross reactivity was seen when the HBsAg ELISA was tested using samples positive for HAV, HCV, HIV, CMV and TP. There was no interference from RF up to 2000 IU/ml. No high dose hook effect up to HBsAg concentrations of 200000 ng/ml.

Limitations:

- Positive results must be confirmed with another available method such as PCR and diagnosis made in conjunction with the other clinical and laboratory information.
- Antigen may be undetectable during the early stages of the disease. Negative results only indicate that the sample does not contain detectable levels of HBsAg and do not rule out the possibility of infection with hepatitis B.
- If, after re-testing of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable and reported as negative.
- Kit failure may result from using kits beyond the expiry date, poor washing procedures, contaminated reagents, improper operation of equipment, sample collection issues or timing errors.
- This kit is intended only for testing of serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled blood.
- This assay is a qualitative assay and the results cannot be used to measure antigen concentration.

References:

1. Stevens,C.E., P.E Taylor and M.J Trong, 1988. Viral hepatitis and liver disease. Alan R Riss, New York.
2. Bhatnagar, P.K., E.Papas, H.E Blum, D.R.Milich, D.Nitecki, M.J Karels and G.N,Vyas 1982. Immune response to synthetic peptide analogues of hepatitis B surface antigen specific for a a determinant. Proc Natl,Acad USA.
3. Szumness,W., C.E.Stevens, E.J Harley, E.A.Zhang, Q.R.Olesko, D.C.Williams, R.Sadovsky, J.M. Morrison and A. Kellner, 1980. Hepatitis B Vaccine: demonstaration of efficacy in a controlled trial in the high risk population in the U>S N.Engl J Med. 303:833-841.

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

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