HBsAg Elisa

CAT NO	DESCRIPTION	PACK SIZE
REF EIAHBH1	HBsAg Elisa IVD	96 Tests
REF EIAHBH2	HBsAg Elisa IVD	480 Tests

Intended Use:

This high sensitivity HBsAg kit 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 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 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.

For detection of HBsAg, this kit uses antibody "sandwich" ELISA method in which, polystyrene microwell strips are pre-coated with monoclonal antibodies specific to HBsAg. Patient's serum or plasma sample is added to the microwells. During incubation, the specific immunocomplex formed in case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum proteins, second antibody conjugated the enzyme horseradish peroxidase (the HRP-Conjugate) and 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. After washing to remove unbound HRP-conjugate, 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 colourless Chromogens are hydrolyzed by the bound HRP-conjugate to a blue-coloured product. The blue colour turns yellow after stopping the reaction with sulfuric acid. The amount of colour intensity can be measured and it is proportional to the amount of antigen captured in the wells, and to its amount in the sample respectively. Wells containing samples negative for HBsAg remain colourless.

Reagent Composition:

COMPONENT	Code	SIZE	DESCRIPTION
Microwell Plate	5	1x96 wells (12x8 well plate) 5x96 wells	Each microwell is coated with antibodies to HBsAg. (Anti HBs). 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 *Preserv.0.1% proClin™ 300*	8	1x1ml 3x1ml	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 *Preserv.0.1% proClin™ 300*	7	1x1ml 3x1ml	Protein stabilized buffer dilution of HBsAg. Red coloured solution. Once open, stable for 1 month at 2-8°C.
Specimen Diluent *Preserv.0.1% proClin™ 300*	9	1x5ml 5x5ml	Green coloured vial containing Buffer solution with protein. Ready to use as supplied. Once open stable for 4 weeks at 2-8°C.
HRP-Conjugate *Preserv.0.1% proClin™ 300*	6	1x6ml 5x6ml	Red coloured liquid. HRP conjugated anti-HBs antibodies. Once open, stable for one month at 2-8°C.
Wash Buffer (20X) *Detergent Tween-20*	1	1x30ml 2x100ml	PBS at pH 7.4. 20X concentrate. Once diluted, stable for 1 week at room temperature, or for 2 weeks when stored at 2-8°C.
Chromogen A	2	1x6ml 1x60ml	Urea peroxide solution. Ready to use. Once open, stable for one month at 2-8°C.
Chromogen B	3	1x6ml 1x60ml	TMB Solution. Ready to use. Once open, stable for one month at 2-8°C.
Stop Solution	4	1x6ml 1x60ml	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 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 450/600~650nm 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 Prestige HBsAg 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 freeze 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 450/600~650nm.**
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 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. 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 Prestige representative.**


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


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

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 3 wells as Negative controls (e.g. B1,C1,D1), 2 wells as Positive controls (e.g. E1,F1) and one Blank (e.g.A1 – taking care that neither the HRP

conjugate nor any samples should be added to the blank well). If the results are read using a plate reader having dual wavelength (450 / 600~650nm) then the Blank well need not be used. Use the required number of strips for the test.

STEP 2

Adding Diluent: Add 20ul of Specimen Diluent into each well except in Blank.

STEP 3

Addition of the sample: Add 100ul 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).

STEP 4

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

STEP 5

Addition of the HRP Conjugate: At the end of the incubation period, remove and discard the plate cover. Add 50ul of HRP conjugate into each well except the Blank, and mix by tapping the plate gently.

STEP 6

Incubation: Cover the plate with the plate cover and incubate for 30 minutes at 37°C.

STEP 7

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 8

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 30 minutes avoiding light. The enzymatic reaction between the chromogen solutions and the HRP conjugate produces blue colour in Positive control and HBsAg Positive samples.

STEP 9

Stopping the Reaction: Add 50ul of the Stop solution into each well and mix gently. Intensive yellow colour develops in the positive control and HBsAg positive sample wells.

STEP 10

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 600~650nm. 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. The results are calculated by relating each specimen absorbance (A) with 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 from the absorbances of the specimens and the controls. In case the results are based on a dual filter plate reader, do not subtract the blank well A value from the specimen and controls absorbances.

Calculation:

Cut off value (C.O) = Nc + 0.06

(Nc = the mean absorbance value for 3 negative controls)

Validation:

Blank well: the absorbance must be <0.080 at 450nm.

Positive Control: the absorbance must be >/= 0.800 at 450/600~650nm or at 450nm after blanking.

Negative control: the absorbance must be </=0.100 at 450/600~650nm or at 450nm after blanking.

If one of the Negative control absorbances does not match the above criteria, this value should be discarded 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 must be re-tested.

Example:

Blank Value	A1: 0.025	450nm (blanking is required only when reading with a single filter)	
Negative control	B1	C1	D1
	0.020	0.012	0.016
Positive Control	E1	F1	
	2.421	2.369	

Calculation of Nc: ((0.020+0.012+0.016)/3) = 0.016

Calculation of the cut off: 0.016 + 0.06 = 0.076

Interpretation of the results:

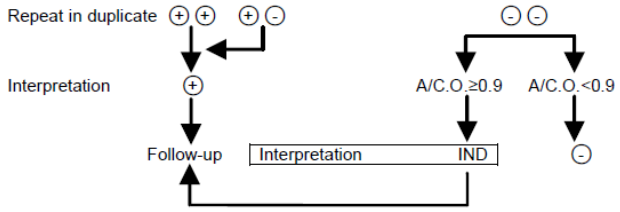
Negative Results: (A/C.O <1) Specimens giving absorbance less than that of the Cut off value are negative for this assay. This indicates that the sample is non-reactive for HBsAg and the patient is probably not infected with HBV and the blood unit does not contain HBsAg and could be transfused in case other infectious diseases markers are also absent.

Positive Results: (A/C.O. >/= 1) Specimens 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 the same kit before final interpretation. Repeatedly reactive specimens can be considered positive for HBsAg.

Borderline: (A/C.O = 0.9 – 1.1) Specimens with absorbance to cut off ratio between 0.9 to 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results.

Follow up, confirmation and supplementary testing of any positive specimen with other analytical systems such as PCR is required. Clinical diagnosis should not be established using a single result.

INITIAL RESULTS INTERPRETATION AND FOLLOW-UP
ALL INITIALY REACTIVE OR BORDERLINE SAMPLES



IND = Indeterminable

- If, after re-testing of the initially reactive samples, both wells show negative results (A/C.O <0.9). These samples should be considered negative and the original result must be classified as false positive. As with many sensitive Elisa Assays, false positive results can occur due to several reasons, most of which are connected with, but not limited to inadequate washing step.
- After retesting in duplicates one or both wells show positive results. The final result of this specimen should be recorded as repeatedly positive. Repeatedly reactive specimens could be considered positive for HBsAg and the blood unit must be discarded.
- After re-testing in duplicates, samples with values close to the cut-off should be interpreted with caution and considered borderline zone sample, or uninterpretable for the time of testing.

Performance Characteristics: Evaluation studies carried out in the Paul-Ehrlich Institut, German Red Cross Institute Baden-Wurttemberg-Hessen have demonstrated the characteristics of the assay.

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

During a multi-centre evaluation, HBsAg Elisa demonstrated a specificity of 99.92%.

Laboratory	Number	HBsAg Elisa		
		Negative	Positive	Specificity
Blood Bank 1	1958	1955	3	99.85 %
Blood Bank 2	2518	2516	2	99.92%
Blood Bank 3	6344	6340	4	99.94%
Total	10820	10811	9	99.92%

Sensitivity: 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 samples available at the Paul-Ehrlich-Institut. With respect to seroconversion sensitivity, the results for 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 HBsAg ELISA detected all positive samples as positive, including the HBV genotypes A-F or HBsAg subtypes examined. In conclusion, the overall score of 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 samples were reactive giving an overall sensitivity of 100%.

Analytical Sensitivity: 0.1 IU/ml (NIBSC 00/588)

Analytical Specificity: No interference was observed with samples 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 samples reactive for anti-HBc, anti-HCV and anti-HIV-1 were screened for HBsAg with this HBsAg ELISA. 98 out of 100 samples were negative for HBsAg. 200

blood samples from patients were also tested with this HBsAg ELISA. 191 out of 200 samples had negative screening results for HBsAg. 8 out of 9 samples with initial reactive screening results had repeat reactive test results with HBsAg ELISA but hepatitis B virus was not confirmed in all cases.

Detection of Mutations:

Type	Description	Total number	This Elisa assay
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:

- Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.
- Antigens may be undetectable during the early stages of the disease. Negative results are only an indication that the sample does not contain detectable levels of HBsAg.
- If, after re-testing of the initially reactive specimens, the assay results are negative, these samples should be considered as non-repeatable and interpreted as negative. As with many sensitive Elisa assays, false positive reactions occur due to several reasons most of which are related to but not limited to inadequate washing step.
- The most common assay mistakes are: using kits beyond expiry dates, bad washing procedures, contaminated reagents, improper operation with equipment, sample collection issues.
- The prevalence of the marker will affect the assay's predictive values.
- This assay cannot be utilized to test pooled plasma. This kit can only be used with individual serum or plasma samples.
- 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. 142. Stevens, C.E., P.E. Taylor, M. J. Tong, P. T. Toy, G.N. Vyas, P. V. Nair.
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.
3. 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
4. Hurie, M.B, E.E Mast and J.P Davis. 1992. Horizontal transmission of hepatitis B virus to U.S born children among refugees, Pediatrics 89: 269-273.
5. 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.
6. 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 determinant. Proc Natl,Acad. Sci. USA 79:4400-4404.

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 Samples	100µl
Incubate	60 minutes
Add HRP-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 450/600~650 nm

EXAMPLE SCHEME OF CONTROLS / SAMPLES 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											

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

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