ImmunoScience INDIA

ImmunoELISA HBsAg

"ELISA Test for the Detection of Hepatitis B surface antigen (HBsAg)"

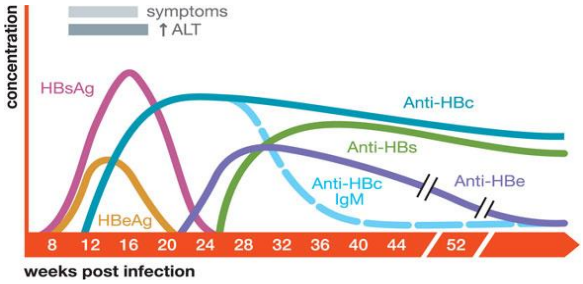
Store at 2 °C to 8 °C

INTENDED USE:

The ImmunoELISA HBsAg is a solid phase enzyme linked immunosorbent assay for the qualitative detection of Hepatitis B surface antigen (HBsAg) in human serum or plasma. It is intended for professional use only as an aid in the identification & diagnosis of infection with Hepatitis B virus. Any reactive specimen with the **ImmunoELISA HBsAg** Kit must be confirmed with alternative testing method(s) and correlate with clinical findings.

SUMMARY OF TEST:

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 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.



PRINCIPLE OF THE TEST:

ImmunoELISA HBsAg Elisa is a solid-phase enzyme-linked immuno-sorbent assay) based on the "sandwich principle". The solid phase of the microtiter plate is made of polystyrene wells coated with mouse monoclonal antibodies specific for HBsAg. 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. Then the second antibody conjugated the enzyme horseradish peroxidase (the HRP-Conjugate) directed against a different epitope of HBsAg is added to the wells.

After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. A color develops in proportion to the amount of antigen bound to Anti-HBsAg mAb coated on the microwells. The peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength of 630.

KIT COMPONENTS (1 X 96 TEST)

1. **Microwell plate (1x96 wells)**: Each well coated with anti-HBsAg monoclonal antibodyin bicarbonate buffer (solvent). The plate is sealed in aluminum pouch with desiccant. The microwell strips can be broken to be used separately.
2. **Negative Control (1x 2.5 ml):** One bottle contains normal human serum and Proclin-300, 0.1% v/v as preservative.
3. **Positive Control (1x 2.5 ml):** One bottle contains inactivated HBsAg human serum and Proclin-300, 0.1% v/v as preservative.
4. **HBsAg specific HRP Conjugate concentrate, 51x (1x 0.6 ml)**: one bottle contains anti-HBsAg –peroxidase conjugate, Bovine serum albumin (as stabilizer) and gentamycin sulphate 0.005% and Proclin-300, 0.05% v/v (as preservative).
5. **Conjugate Diluent (1x 20 ml)**: one bottle contains phosphate saline-casein buffer and Proclin-300 0.05% v/v (as preservative).
6. **TMB Substrate, 101x concentrated (1x0.3 ml)**: one bottle contains tetramethyl benzidine in Dimethyl sulphoxide as solvent.
7. **Substrate Buffer (1x20ml)**: one bottle contains Citrate-Acetate buffer (solvent) containing hydrogen peroxide 0.006% v/v.
8. **Wash Buffer concentrate 20x (1x50ml)**: one bottle contains concentrated phosphate buffered saline with polysorbate (surfactant) and Proclin-300 (preservative), 0.05 %v/v.
9. **Stop Solution (1x20ml)**: one bottle contains 1.5 N sulphuric acid.
10. **Instruction manual/product Insert.**

MATERIALS REQUIRED BUT NOT PROVIDED:

1. Micro pipette capable of delivering 10 µl, 50 µl, 75 µl, and 100 µl volumes with a precision better than 1.5%.
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450 nm wavelength is acceptable.
3. Absorbent paper for blotting the microplate wells
4. Parafilm or other adhesive film sealant for sealing plate
5. Timer
6. Distilled or de-ionized water.

SPECIMEN COLLECTION AND REPARATION:

1. Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
2. This kit is designed for use with serum or plasma specimen without additives only.
3. If a specimen is not tested immediately, the serum/plasma shall be separated and refrigerated at 2°C - 8°C. If storage period greater than three days are anticipated, the separated serum/plasma should be frozen (-20°C).
4. Avoid repeated freezing-thawing of specimens.
5. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
6. Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.
7. Do not use serum specimens demonstrating gross lipemic or lipemia, gross hemolysis or turbidity.
8. Do not use specimens containing sodium azide as preservative.

REAGENT PREPARATION

A. Wash Buffer preparation: Dilute 20x wash buffer provided in the kit in 1:20 ratio in purified water. For example, to prepare 1000 ml wash buffer, mix 50 ml of 20x wash buffer into 950 ml of purified water. Decide the volume of buffer according to the required number of test and dead volume of washer instrument. In case of manual washing, the volume can be calculated accordingly.

B. HRP Conjugate preparation: Dilute HRP conjugate concentrate (51x) in conjugate Diluent (1:51 ratio) as follows:

Strips	1	2	3	4	5	6	7	8	9	10	11	12
HRP C. 51x (µl)	20	40	60	80	100	120	140	160	180	200	220	240
Conj. Diluent (ml)	1	2	3	4	5	6	7	8	9	10	11	12

C. TMB substrate preparation: Dilute TMB substrate concentrate (101x) in Substrate Buffer (1:101 ratio) as follows:

Strips	1	2	3	4	5	6	7	8	9	10	11	12
TMB S. 101x (µl)	10	20	30	40	50	60	70	80	90	100	110	120
Substrate Buffer (ml)	1	2	3	4	5	6	7	8	9	10	11	12

- ✓ Prepare the reagents just before use.
- ✓ Prepare only the required quantity of reagents.
- ✓ Ensure the TMB substrate to be kept in low light and closed container.
- ✓ Discard the remaining reagent after use.

ASSAY PROCEDURE

1. Take the required number of strips and fix them to plate.
2. Pipette 50 µl of Negative control into each well from 1A to 1C and 50 µl of Positive Control into each well from 1D to 1E, respectively and then, pipette 50 µl of each specimen into the remaining well.
3. Add 100 µl of diluted HRP conjugate in each well.
4. Mix the added samples manually or by using microplate shaker, take care not to mix or splash contents out of well while mixing.
5. Seal the microwell strips using the plate cover and incubate at 37°C ± 1°C for 60 minutes.
6. Before 5 to 10 minutes of completion of the 1st incubation, make 1:101 dilution of TMB substrate with substrate buffer.
7. Aspirate the contents from all the wells and wash 5 times with 325 µl of diluted washing solution. (325 µl/well/time)
8. Invert the plate and tap it on absorbent paper to remove the remaining wash solution, and then, pipette 100 µl of prepared diluted substrate into each well. And incubate at controlled room temperature (21°C - 25°C) in dark environment for 15 minutes.
9. Pipette 100 µl of stop solution into each well and tap the plate gently to homogenize the coloring materials.
10. Read the absorbance at 450 nm (reference wavelength at 630 nm) against an air blank within 30 minutes after pipetting of stop solution.

QUALITY CONTROL

1. The average absorbance of Positive Control (PCx) should be greater than or equal to 1.0.
2. The average absorbance of Negative Control (NCx) should be less than or equal to 0.200.

INTERPRETATION OF RESULTS

(a) Calculate the negative control mean(NCx)
Ex. Negative Control 1 absorbance=0.045
Negative Control 2 absorbance=0.050
Negative Control 3 absorbance=0.055
Negative Control Mean (NCx)=
(0.045+0.050+0.055)/3=0.050

(b) Calculate the positive control mean (PCx)
Ex. Positive Control 1 absorbance=2.13
Positive Control 2 absorbance=2.05
Positive Control Mean (PCx)= (2.13+2.05)/2=2.09

(c) Calculate the cut off value
Cut off value= NCx + 0.200

INTERPRETATION

Samples with absorbance greater than or equal to the Cut Off value are considered positive to HBSAg. Samples with absorbance less than Cut Off value are considered negative to HBsAg.Sample values within the ±10% of cut off value should be considered as indeterminate or grey zone samples. It should be retested again or repeat with freshly collected sample.

WARNING AND PRECAUTIONS:

1. This product is made for in Vitro Diagnostic Use only.
2. Package insert must be read completely before performing the test. Failure to follow the instructions in pack insert may give inaccurate test results.
3. Do not use the Kit beyond the expiry date.
4. Bring all reagents & samples to room temperature (18°C-28°C) before use.
5. Do not use the components in any other type of test kit as a substitute for the components in this kit.
6. Do not use hemolyzed blood specimen for testing.
7. Do not ingest the reagents. Avoid contact with eyes, skin and mucose. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
8. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
9. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
10. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
11. In the beginning of each incubation and after adding Stop Solution, gently rocking the microwells to ensure thorough mixing. Avoid the formation of air bubbles as it may give inaccurate absorbance values. Avoid splash liquid while rocking or shaking the wells.
12. Don't allow the micro plate to dry between the end of the washing operation and the reagent distribution.
13. The enzyme reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or substrate solution.
14. The TMB substrate solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The TMB Substrate must be stored in the dark.
15. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.

16. The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.
17. Avoid strong light or sunlight during color development.

LIMITATIONS:

1. The **ImmunoELISA HBsAg** is intended to detect antigen against HBsAg. However, the test cannot detect the quantity or relative change in the levels of antibodies.
2. The product is not made for detection of HBsAg Antibody.
3. The product is designed so as to give best results but, some samples may show cross reactivity. Hence, there may be a chance of false positive results. Every positive result shall be verified by testing with alternate kit or methods and also shall be correlated with symptoms.

REFERENCES:

1. Kim, C. Y., Tillis, J. G. 1973, Purification of Biophysical characterization of Hepatitis A antigen, J. Clin. Invest, 52, May 1973, Pgs. 1176-1186.
2. Kee Myung Lee et.al., Emergence of Vaccine- induced escape mutant of Hepatitis B Virus with Multiple surface gene mutations in a Korean child, J.Korean. Med.Sci., 2001, 16, Pgs 356-361.
3. Koyanagi T et al. Analysis of HBs antigen negative variant of hepatitis B virus: Unique Substitutions, Glu 129 to Asp and Gly 145 to Ala in the surface antigen gene. Med Sci Monit, 2000; 6(6): Pgs1165-1169.

IVD	In Vitro Diagnostic Use
	Manufacturer
	Manufacturing Date
	Expiry Date
LOT	Lot Number
	Store at + 2°C to + 8°C
	Single Use
	Number of tests in the pack
	Do not use if pouch or kit damaged
	This side Up
	Read package insert beforeuse

MANUFACTURED BY

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