Hepatitis B
Surface Antigen (ELISA)
(For in-vitro diagnostic use only)
Store at 2 to 8°C

INTENDED USE
The Hepatitis B Surface Antigen (HBsAg) EIA Kit is an enzyme immunoassay kit on microstrip format for the qualitative detection of HBsAg in human serum or plasma samples. Unknown specimens non reactive with the ATLAS HBsAg EIA are negative for HBsAg. Specimens giving a positive reaction should be retested and confirmed by a neutralization test.

INTRODUCTION
Hepatitis B is a disease caused by viral infection. The route of infection can be improper needle puncture, blood transfusion or even by taking contaminated food or water. Hepatitis B has become a significant problem for public health management. Almost one in every ten adults, who have been infected by Hepatitis B Virus (HBV), develops some form of chronic liver disease and becomes a long-term carrier of HBV. Screening for Hepatitis B is therefore urgently needed. Hepatitis B is an immune disease. Invasion of the human body by HB virus induces autoimmune reactions, which damage the liver. The components of the virus (antigens) and the host responses (antibodies), the so-called immunologic markers have often been used as diagnostic tools.

There are six immunologic markers of HBV: HBsAg, HBeAg, and their respective antibodies. The HBsAg however is the first marker to appear in serum. The presence of HBsAg indicates recent infection and if it persists for more than 6 months the patient may become a chronic carrier.

PRINCIPLE
ATLAS HBsAg adopts the "sandwich principle" as the basis of the assay. When a positive sample is incubated into the well, the coated monoclonal antibody (solid phase antibody) binds the HBsAg of the sample. A further incubation with a second monoclonal anti-HBsAg labelled with the enzyme peroxidase (anti-HBsAg-HRP) leads to the sandwich immunocomplex bound onto the well: solid phase antibody: HBsAg: Antibody-HRP. The activity of the HRP is then revealed by the addition of the substrate, which becomes blue and then turns to yellow after blocking the reaction with acid.

MATERIALS
MATERIALS PROVIDED
- Coated microplates: one microtiter plates with 96 wells, coated with mouse monoclonal antibody against HBsAg (8 removable strips with 12wells each). Store at 2-8°C.
- Enzyme Conjugate:
  - One vial (7.5 ml) containing a solution of Anti-HBsAg-HRP conjugate. Store at 2-8°C.
- HBsAg Negative Control:
  - One vial (1 ml) containing human serum negative for HBsAg. Store at 2-8°C.
- HBsAg Positive Control:
  - One vial (1 ml) containing human serum positive for HBsAg. Store at 2-8°C.
- Substrate Solution (A)
  - One vial (7.5 ml) containing Urea Hydrogen Peroxide. Store at 2-8°C.
- Substrate Solution (B)
  - One vial (7.5 ml) containing stabilized 3,3’-5,5’ Tetramethylbenzidine (TMB). Store at 2-8°C.
- Stop Solution
  - One vial (7.5 ml) containing 1.0 M H2SO4. Ready to use.
- Wash Buffer (20X Conc.)
  - One vial (30 ml) containing Phosphate Buffered Saline.
- Package insert.
- 1 place of plate lid.
- 1 zip-lock bag.

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED
- Precision Micropipettes and tips (50 µl).
- Incubator (+37°C).
- Automatic plate washer.
- Microtiter plate reader for the measurement of absorbance at 450 nm.
- Adsorbent pad or paper.
- Distilled water.
- Vortex mixer.

PRECAUTION
- The Positive Control is made of HBsAg positive sera and has been heated at 60°C for 10 hours. This is a generally accepted method to inactivate the Hepatitis agent. However, for safety precautions, it must be treated as potentially infectious material. Both positive and negative controls have been tested and found negative for HIV 1-2 and Hepatitis C virus. However, they must be treated as potentially infectious agents.
- Do not smoke or eat where specimens or reagents of the kit are handled.
- Do not pipette by mouth. Wear PVC gloves when handling reagent kits or specimens and wash hands thoroughly afterwards.
- Non acid containing spills should be wiped up thoroughly with 5% sodium hypochlorite solution.
- All waste material should be properly disinfected before disposal. Both liquid and solid waste material can be autoclaved for one hour at 121°C. Solid waste material also be incinerated. Non-acid waste material can be treated with sodium hypochlorite (bleach solution) diluted to a final concentration of 1.0%. Acid liquid waste material requires neutralization before similar treatment and should stand for 30 minutes to obtain effective disinfections.

SPECIMEN COLLECTIONS AND STORAGE
- Either serum or plasma can be used with this diagnostic kit.
- Whole blood specimens should be separated from red blood cell as soon as possible in order to avoid hemolysis.
- Also clots must be removed.
- Specimens must be refrigerated at 2°C to 8°C before testing to minimize deterioration.
- For long-term storage they should be frozen below -20°C.

PREPARATION OF WORKING WASHING SOLUTION
1. Into a graduated peaker of at least 800ml capacity, pour the entire contents of the concentrated wash solution(30 ml).
2. Rinse the vial with distilled water and pour also this distilled water into the peaker up to a total volume of 600ml.
3. Transfer the contents of the peaker into a 1000 ml bottle for storage.

**PROCEDURE**

1. Bring all reagents and specimens to room temperature (15°C-30°C) before beginning the assay. Swirl gently before use. Adjust the incubator at +37°C, if necessary.
2. Write down the relative numbers of specimens and wells on the data sheet. One well for the blank, five additional wells for the controls and one well for each specimen. If the experiment does not need the whole plate, remove the remaining strips from the strip holder and store the unused strips in the ziplock bag (included in the kit) which contains desiccant.
3. Reserve one well for blank, add 50ul of the Negative Control to each of three wells, 50ul of the Positive Control to each of two wells and 50ul of specimen to respective wells, then Pipette 50ul of Anti-HBsAg peroxidase-conjugate working solution into each well, except the blank (1A) according to the following scheme:
   
   1A : Blank  
   2A , 3 A,4 A : Negative Control Serum  
   5 A, 6 A : Positive Control Serum  
   7 A : Samples

**Notes:**
- use an individual tip for each pipetting to avoid cross contaminations.
- If more than 2 or 3 runs per plate are required and reliable apparatus (i.e. pipettes, washers, etc.) are used, one can pipette the Negative Control in duplicate and the Positive control in singleton.

4. Incubate the plate in a +37°C incubator for 30 minutes.
5. At the end of the incubation wash the strips 6 times with the working wash solution either manually or with an automatic washer. After the final washing, make sure that whole solution is perfectly removed from each well.

**Note:**

**Proper washing procedure is essential for good assay performance.**

6. Dispense 50ul of Substrate solution A and B, including 1A. Mix horizontally and incubate for 10 minutes at +37°C (avoid exposure to direct sun light).
7. Stop the reaction by adding 50ul of Blocking Reagent to each well (including the blank) in the same order adopted for the addition of the substrate solution.
8. After adding the stop solution, read the color developed on the microplate reader at 450 nm. The reading should be done within 30 min. from the stop.

**Note:**

The reader should be blanked at 450 nm against the blank. Bichromat absorbance measurement with a reference wavelength of 620nm is recommended when available.

**WASHING PROCEDURE**
- Microtiter plates could be washed manually or automatically, in which case it is absolutely necessary to program the microplate washer according to the following directions:
  - Aspirate the incubation mixture from the wells of the first strip and dispense 350 ul of washing solution. Soon afterwards process the other strips in the same way. Repeat this procedure for 5 further times, for a total of 6 cycles, making sure that the washing solution remains in the wells for 30 sec. (soaking time) each cycle. Proper wash procedure is essential for a good assay performance.

**Example:** NCx = 0.032  
Cut-off = 2.1 * 0.05 = 0.105
- Any sample, in which the absorbance is equal to or higher than the Cut-off value, is considered Positive for HBsAg. Any sample, in which the absorbance is lower than the calculated Cut-off value, is considered Negative for HBsAg.

**SENSITIVITY**
Sensitivity: 0.5ng/ml

**SPECIFICITY**
Specificity: > 99.5
Both Ad and Ay subtypes are detected.

**LIMITATION OF THE PROCEDURE**
- The user of this kit is advised to carefully read and understand the package insert.
- Strict adherence to the protocol is necessary to obtain reliable test results, in particular, correct sample and reagent pipetting, along with careful washing and timing of incubation steps are essential for an accurate, reproducible detection of HBsAg.
- Use fresh plasma samples, or samples frozen and thawed only once. Samples degradation as well as multiple freezes - thaw cycles may cause erroneous results. Do not use heat-inactivated samples.

**REFERENCE**

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