For Research Use Only *Not for Diagnostic Use*

HEPATITIS B – HBsAg

Catalog #: WB2296

SURFACE ANTIGEN (HBsAg) ELISA
One-Step Incubation, Double Antibody Sandwich Principle

INSTRUCTIONS FOR USE

This kit is an enzyme-linked immunosorbent assay (ELISA) for qualitative detection of HBsAg in human serum or plasma. For research use only.

SUMMARY

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.
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.

PRINCIPLE OF THE ASSAY

This HBsAg ELISA kit uses polystyrene microwell strips pre-coated with monoclonal antibodies specific to HBsAg. Patient's serum or plasma sample is added to the microwell together with a second antibody conjugated with horseradish peroxidase (HRP) and directed against a different epitope of HBsAg. 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 and unbound HRP-conjugate, Chromogen solutions containing tetramethylbenzidine (TMB) and urea peroxide are added to the wells. In presence of the antibody-antigen-antibody(HRP) "sandwich" immunocomplex, 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 can be measured and is proportional to the amount of antigen in the sample. Wells containing samples negative for HBsAg remain colorless.

COMPONENTS

96 Tests (12 wells)

- **MICROWELL PLATE** 1 plate

Blank microwell strips fixed on white strip holder.

Eight 12-well strips per plate. Each well contains monoclonal antibodies reactive to HBsAg (anti-HBs). The plate is sealed in aluminium pouch with desiccant.

The microwell strips can be broken to be used separately. Place unused wells in the plastic sealable storage bag together with the desiccant and return to 2~8ºC.

- **NEGATIVE CONTROL** 1 vial

Yellowish liquid filled in vial with green screw cap

1 ml per vial

Protein-stabilized buffer tested non-reactive for HBsAg.

Preservatives: 0.1% ProClin 300.

Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **POSITIVE CONTROL** 1 vial

Red color liquid filled in vial with red screw cap.

1ml per vial

HBsAg diluted in protein-stabilized buffer containing preservatives: 0.1% ProClin 300.

Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **HRP-CONJUGATE REAGENT** 1 vial

Red liquid filled in a white vial with red screw cap.

7 ml per vial

Horseradish peroxidase-conjugated anti-HBs antibodies

Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **STOCK WASH BUFFER** 1 bottle

Colorless liquid filled in blank bottle with white screw cap.

30 ml per bottle

PH 7.4 20 × PBS (Containing Tween-20 as a detergent).

DILUTE BEFORE USE -The concentration must be diluted **1:19** with distilled/deionized water before use.

Once diluted, stable for one week at room temperature or for one month at 2-8ºC.

- **CHROMOGEN SOLUTION A** 1 vial

Colorless liquid filled in white vial with green screw cap.

7 ml per vial

Urea peroxide solution.

Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **CHROMOGEN SOLUTION B** 1 vial

Colorless liquid filled in the black vial with black screw cap.

7 ml per vial

TMB solution. Tetramethylbenzidine dissolved in citric acid.

Ready to use as supplied. Once open, stable for one month at 2-8ºC.

- **STOP SOLUTION** 1 vial

Colorless liquid filled in white vial with yellow screw cap

7 ml per vial

Diluted sulfuric acid solution (2.0M H_2SO_4).

- **PLASTIC SEALABLE BAG** 1 unit

For enclosing the strips not in use.

- **CARDBOARD PLATE COVER** 1 sheet

To cover the plates during incubation and prevent evaporation or contamination of the wells.

- **PACKAGE INSERTS** 1 copy

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED BUT NOT PROVIDED

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Disposable V-shaped troughs.
5. Dispensing system and/or pipette (single or multichannel), disposable pipette tips
6. Absorbent tissue or clean towel.
7. Dry incubator or water bath, 37±0.5ºC.
8. Microshaker for dissolving and mixing conjugate with samples.
9. Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
10. Microwell aspiration/wash system.

SPECIMEN COLLECTION，TRANSPORTATION AND STORAGE

1. **Sample Collection**: Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM (round per minutes) for 20 minutes at room temperature (18-30ºC)or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they can give false results in the assay. Do not heat inactivate samples. This can cause sample deterioraration.
2. **Transportation and Storage:** Store samples at 2-8ºC. Samples not required for assay within 3 days should be stored frozen (-20ºC or lower). Avoid multiple freeze-thaw cycles.

SPECIAL INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles of 350-400µl/well are sufficient to avoid false positive reactions and high background.
3. To avoid contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. 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.
5. In case of manual washing, we suggest to carry out at least 5 cycles, dispensing 350-400µl/well and aspirating the liquid for 5 times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips must be treated with a sodium hypochlorite solution at a final concentration of 2.5% for 24 hours, before liquids are wasted in an appropriate way.
7. The concentrated Washing solution must be diluted 1:19 before use. For one plate, mix 30 ml of the concentrate with 570 ml of water. If less than a whole plate is used, prepare the proportional volume of solution.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8 ºC, **do not freeze**. To assure maximum performance of this HBsAg ELISA kit, protect the reagents from contamination with microorganism or chemicals during storage.

PRECAUTIONS AND SAFETY

This kit is intended **FOR RESEARCH USE ONLY**

The ELISA assay is time and temperature sensitive. To avoid incorrect result, strictly follow the test procedure steps 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 all the 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. Allow the reagents and samples to reach room temperature (18-30ºC) before use. Shake reagent gently before use.
4. Return to 2-8ºC immediately after use.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. 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.
8. Avoid assay steps long time interruptions. Assure same working conditions for all the wells.
9. Calibrate the pipette frequently to assure the accuracy. Use different disposal pipette tips for each specimen and reagents in order to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes and disposable tips is recommended.
11. Assure that the incubation temperature is 37 ºC inside the incubator
12. When adding samples avoid touching the well's bottom with the pipette tip.
13. When reading the absorbance with a plate reader, it is recommended to determine the absorbance at 450nm and with reference at 630nm.
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. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
15. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121ºC or treated with 10% sodium hypochlorite for 30 minutes to decontaminate before any further steps for disposal.
16. The Stop solution 2M H_2SO_4 is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
17. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.

ASSAY PROCEDURE

Step 1 **Reagents preparation:** Allow the reagents to reach room temperature (18-30°C). Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37ºC until crystals dissolve. Dilute the stock Wash buffer 1:19 with distilled or deionized water. Use only clean vessels to dilute the buffer.

Step 2 **Numbering Wells:** Set the strips needed in strip-holder and number sufficient number of wells including three Negative control **(e.g. B1, C1, D1)**, two Positive control **(e.g. E1, F1)** and one Blank (**A1**, Neither samples nor HRP-Conjugate should be added into the Blank well). Use only number of strips required for the test.

Step 3 **Adding Sample and HRP-Conjugate**: Add **50 µl** of Positive control, Negative control, and specimen into their respective wells. **Note: Use a separate disposal pipette tip for each specimen, Negative Control and Positive Control to avoid cross-contamination.** Add **50 µl HRP-Conjugate** to each well except the Blank and mix by tapping the plate gently.

Step 4 **Incubating:** Cover the plate with the plate cover and incubate for **60 minutes at 37°C**. It is recommended to use water tank to assure the temperature stability and humidity during incubation. If dry incubator is used, do not open the door frequently.

Step 5 **Washing:** At the end of the incubation, remove and discard the plate cover. Wash each well **5** times with diluted Wash Buffer. Each time allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the strips plate down onto blotting paper or clean towel, and tap the plate to remove any remainders.

Step 6 **Coloring:** Dispense **50µl** of Chromogen A and **50µl** Chromogen B solution into each well including the **Blank**, and mix by tapping the plate gently. Incubate the plate at **37ºC for 30 minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and HBsAg Positive sample wells.

Step 7 **Stopping Reaction:** Using a multichannel pipette or manually add **50 µl** Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and HBsAg Positive sample wells.

Step 8 **Measuring the Absorbance:** 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:** read the absorbance within **5** minutes after stopping the reaction)

INTERPRETATION OF RESULTS AND QUALITY CONTROL

Each microplate must be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on Single filter plate reader, the results must be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on Dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

1. Calculation of Cut-off value
Cut-off value (C.O.) = *NC X 2.1

*NC = the mean absorbance value for three negative controls.

Example:
1. Calculation of NC:

Well No	B1	C1	D1	
Negative Controls OD value	0.02	0.012	0.016	Nc=0.016

2. Calculation of Cut-off value:C.O.= 0.016+0.05 = 0.066

Quality Control Range.

Note: If the optical density (OD) value of ONE of the NEGATIVE CONTROLS is ABOVE the stated quality control range, it should be discarded and the mean value is calculated again using the remaining two values. If more than one NEGATIVE CONTROL OD value does not meet the Quality Control Range specifications, the test is invalid and must be repeated.

2. Quality control Range.

The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed

a) The absorbance of the Blank well is less than 0.08 at 450.

b) The absorbance value OD of the Positive control must be equal to or greater than 1.800 after blanking.

c) The absorbance value OD of the Negative control must be less than 0.100 after blanking.

3. Interpretations of results:

(S = the individual absorbance (OD) of each specimen)

- **Negative Results (S/C.O. <1)**: samples giving an absorbance less than the Cut-off value are considered negative, which indicates that no hepatitis B surface antigen has been detected with this HBsAg ELISA kit
- **Positive Results (S/C.O.≥1)**: samples giving an absorbance greater than or equal to the Cut-off value are considered initially reactive, which indicates that HBV surfaces antigen has probably been detected with this HBsAg ELISA kit
- **Borderline:** Samples with absorbance to Cut-off ratio between 0.9 and 1.00 are considered borderline samples and retesting is recommended. Repeatedly positive samples can be considered positive for HBsAg.

LIMITATIONS

1. Non-repeatable positive result can occur due to the general biological and biochemical characteristics of ELISA assays. The test is designed to achieve performance characteristics of high sensitivity and specificity. However, in very rare cases some HBV mutants or subtypes may remain undetectable. Antigens may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. Any positive result must be interpreted in conjunction with patient clinical information and other laboratory testing results.
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.

VALIDITY

As indicated in the labellings. Never use this kit Beyond the Expiration.

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XpressBio
Life Science Products

P.O. BOX 458
Thurmont, MD 21788 USA
Tel: 301-228-2444 Fax: 301-560-6570
Toll Free: 888-562-8914
www.xpressbio.com info@xpressbio.com