

HBsAb ELISA Kit

Enzyme-linked immunosorbent assay for the detection antibody against HBsAg in serum or plasma

Catalog #:CT1002B

INTENDED USE

HBsAb EIA is a qualitative enzyme immunoassay for the detection of antibody to HBsAg in human serum or plasma.

SUMMARY AND PRINCIPLE OF THE TEST

Hepatitis B surface Antigen (HBsAg) is the major structural polypeptide of the envelope of the Hepatitis B Virus (HBV). This antigen is composed mainly of the type common determinant "a" and the type specific determinants "d" and "y", present only on the specific serotypes. Upon infection, a strong immunological response develops firstly against the type specific determinants and in a second time against the "a" determinant. Anti "a" antibodies are however recognised to be most effective in the neutralisation of the virus, protecting the patient from other infections and leading it to convalescence. The detection of HBsAb has become important for the follow up of patients infected by HBV and the monitoring of recipients upon vaccination with synthetic and natural HBsAg.

Anti-HBsAg titer can be determined to monitor the prognosis of patients recovering from the hepatitis B viral infection. It also can be used as an indicator of prior exposure to Hepatitis B viruses.

The HBsAb EIA is a solid-phase simultaneous immunoassay to detect antibody against HBsAg. Microwells are coated with HBsAg. A serum specimen is added to the microwells together with Horseradish Peroxidase (HRP) conjugated HBsAg. After incubation, the complex of antigen-antibody-antigen (HRP-conjugated HBsAg, anti-HBsAg antibody and HBsAg on the wells) will be formed. Thus, the amount of HRP-HBsAg bound to the well is proportional to the concentration of anti-HBsAg antibody in the specimen.

The unbound enzyme conjugates will be washed away and then the chromogen substrate solution containing urea peroxide is added to the wells. A blue color is developed in proportion to the amount of anti-HBsAg antibody in the specimens. The enzyme-substrate reaction is stopped by the addition of sulfuric acid. The absorbance of controls and specimens is determined by using EIA reader with wavelength set at 450 nm.

REAGENTS

Materials provided with the kits:

1. Microtiter Well: coated with natural inactivated HBsAg antigen.
2. Negative Control: 0.25ml normal serum.
3. Positive Control: 0.25ml anti-HBsAg positive serum.
4. Enzyme Conjugate: 6 ml, HBsAg-HRP conjugate.
5. Wash Buffer Concentrate (20x): 25 ml, The buffer should be diluted 20 times with distilled water before use.
6. Substrate Solution A: 6 ml Urea Peroxide
7. Substrate Solution B: 6 ml TMB solution
8. Stop Solution: 6 ml 2N Sulfuric Acid

Materials required but not provided:

1. Precision pipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Plasma samples containing EDTA, heparin or oxalate may interfere with test procedures and should be avoided. Specimen with extensive particulate should be clarified by centrifugation prior to use. Covered specimens may be stored for up to 48 hours at 2°-8°C prior to assaying. Specimens held for a longer time can be frozen at -20°C for mix prior to testing. Avoid repeated freeze thaw. At least, two wells of negative and positive controls each should be run in every assay.

PRECAUTIONS

1. Caution: Some components of this kit contain human serum. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
2. Wear disposable gloves while handling kit reagents and specimens and thoroughly wash hands afterwards.
3. Dispose off all specimens and materials used to perform the test as if they contained infectious agents.
4. Do not mix reagents from kits with different lot numbers.
5. Cross contamination between reagents will invalidate the test results.
6. All reagents and components except the conjugate must be equilibrated at room temperature prior to use.

STORAGE OF TEST KITS AND INSTRUMENTATION

Unopened test kits should be stored at 2°-8°C upon receipt. Micro titer plate, once opened, should be kept in a sealed bag with desiccants to minimize exposure to damp air. To remove the required number of strips from the micro titer plates, bring the sealed pouches to room temperature first and then open the pouches. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. Opened test kits will remain stable until the expiration date shown in 4°C, provided it is stored as described above. A micro titer plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

WORKING REAGENT PREPARATION, STORAGE AND STABILITY

No reagent preparation is required except for wash buffer, which is supplied as a 20 X concentrate.

WORKING WASH BUFFER

Dilute the 20X wash buffer concentrate with deionized or distilled water 1:20. For example, 5 ml of wash buffer concentrate should be diluted to a total volume of 100 mL with deionized or distilled water.

STABILITY OF OPENED KIT COMPONENTS AND DILUTED REAGENTS

The diluted wash buffer is stable for at least one week when stored at room temperature. Substrate is stable for the expiration date of the kit. The micro titer plates should be opened after they have been kept at room temperature for 20-30 minutes. After removing the required number of strips, the plates should be resealed in the foil pouch bags along with the desiccant and stored at 2°-8°C. Exposure of HBsAg plates to humidity drastically reduces the shelf life.

ASSAY PROCEDURE:

It is strongly advised to analyze each specimen and controls in duplicate. All the reagents should equilibrate to room temperature before use.

1. Dispense one drop (50 ul) of Positive Control as well as Negative Control in duplicate into respective wells. Set one blank well as background control, and 50ul of serum or plasma samples into respective test wells
2. Add one drop (50 ul) of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 minutes. Do not add Enzyme Conjugate to the blank well.
3. Place the microtiter plates into a humidified box, and incubate at 37°C for 30 minutes.
4. Wash each well 4 times by filling each well with diluted wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
5. Add one drop (50 ul) of Substrate Solution A (HRP-substrate) to each well, then add one drop (50 ul) of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 15 minutes. .
6. Add 1 drop (50 ul) of Stop Solution to each well to stop the color reaction. Read O.D. at 450 nm with an EIA plate reader.

ASSAY VALIDITY

EIA Plate Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells, OD value of Positive Control should be between 1.5-2.5).

INTERPRETATION OF RESULTS

Positive: P/N value is equal to or greater than 2.1

Negative: P/N value is less than 2.1

$$\text{P/N value} = \frac{\text{OD value of specimen}}{\text{Average OD value of Negative Control}}$$

If the OD value of the negative control is less than 0.05, it should be reported as 0.05. If it is more than 0.05, it should be reported as the actual OD value measured.

LIMITATIONS OF THE PROCEDURE

1. HBsAb EIA is limited to the detection of antibody against HBsAg in serum or plasma.
2. As in other sensitive immunoassays, there is the possibility that non-repeatable reaction may occur due to inadequate washing. So do aspirate the well or get rid of the entire content of wells before adding the washing solution.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be made only on the basis of a single test. A complete evaluation by physician is needed for a final diagnosis.

RELATED READING MATERIALS

1. Lander, J et al., Viral hepatitis type B (MS-2 strain). Detection of antibody after primary injection. *New Eng. J. Med.* 285-303 (1971).
2. Polesky H.F. and Olson C. The incidence and significance of antibody to Australia antigen in blood donors. *Am. J. Clin. Pathol* 56:129 (1971).
3. Lander J.J. et al. Antibody to hepatitis associated antigen. *JAMA* 220: 1079-1082 (1972).
4. Yuasa T. et al. Hepatitis B antigen and antibody prevalence of Japanese sera selected from the 1972 year's collection at National serum bank, National Institute of Health of Japan. *Japan J. Med. Sci. Biol* 34:181-190 (1980).
5. Magnius, L.O. et al., A new antigen-antibody system. Clinical significance in long-term carrier of hepatitis B surface antigen. *J. Am. Med. Assoc.* 231: 356-359.
6. Blumberg BS et al., Australia antigen and hepatitis. *New Eng. J. Med.* 283:349-354 (1970).

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November 10, 2007 Revision: 02



In vitro diagnostic device



Lot code



Consult instructions for use



Catalogue number



Keep dry



Contains sufficient for <n> tests



Temperature limitation



Manufacturer



Use by



Do not use if package damaged