

HBsAg ELISA Kit

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

INTRODUCTION

HBsAg ELISA is used for the qualitative determination of Hepatitis B surface antigen (HBsAg) in human serum or plasma. This test is indicated for the screening of blood and blood products to be used for transfusion and an aid for the diagnosis of existing or previous hepatitis B infection.

INTRODUCTION

HBsAg is one of the earliest markers that appear in the blood following infection with Hepatitis B virus (HBV). This infection of the liver is transmitted by homosexual or heterosexual activity, blood borne exposure, mother - infant, close personal contact and by intake of contaminated water and food products. In the HBV infected people, the virus persists for the rest of their lives and can be passed on to others. Therefore Hepatitis B has become a global public health problem.

Infection with HBV results in the appearance of a number of serological markers and one of the first of such markers is Hepatitis B surface antigen (HBsAg). The HBV infection causes a wide variety of liver damages such as acute self-limiting infection, fulminating hepatitis, chronic hepatitis with progression to cirrhosis and liver failure, and a symptomatic chronic carrier state.

Hepatitis B surface antigen (HBsAg) appears 1-7 weeks before biochemical evidence of liver disease or jaundice. Three weeks after the onset of acute hepatitis almost half of the patients will still be positive for HBsAg. In the chronic carrier state, the HBsAg persists for long periods (6-12 months) with no seroconversion to the corresponding antibodies. Therefore, screening for HBsAg is highly desirable for all donors, pregnant women and people in high-risk groups.

PRINCIPLE OF THE TEST

The HBsAg EIA is a solid-phase simultaneous sandwich immunoassay, which employs monoclonal antibodies and polyclonal antibodies specific for HBsAg. Microtiter well are coated with monoclonal antibodies specific for HBsAg. A serum specimen is added to the antibody coated Microtiter wells together with enzyme conjugated polyclonal antibodies. HBsAg, if present, will form an antibody-HBsAg-antibody-enzyme complex. The plate is then washed to remove unbound material. Finally, a solution of substrate is added to the wells and incubated. A blue color will develop in proportion to the amount of HBsAg present in the specimen. The enzyme-substrate reaction can be stopped and the result is visualized by naked eye or read by EIA plate reader for absorbance at the wavelength of 450 nm.

REAGENTS

Materials provided with the kits:

1. Microtiter Well coated with monoclonal anti-HBs antibody: 96X5 tests.
2. Negative Control: One vial of 1.5ml HBsAg Negative Control.
3. Positive Control: One vial of 1.5ml HBsAg Positive Control.
4. Enzyme Conjugate: 30 ml containing HRP-conjugated-anti-HBs for 96 tests.
5. Wash Buffer Concentrate (20 x): . 125 ml for 480tests. The buffer should be diluted 20 times with distilled water before use.
6. Substrate Solution A: 30 ml HRP Substrate for 480 tests.
7. Substrate Solution B: 30 ml TMB Chromagen Substrate for 480tests.
8. Stop Solution: 30 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

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

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. HBsAg kit is used for the detection of HBsAg in human serum or plasma. Based on a single reactive test result, a sample should not be considered HBsAg positive. Further testing, including confirmatory testing, should be performed before a specimen is considered positive for HBsAg. A non-reactive test result does not exclude the possibility of exposure to hepatitis B virus. Levels of HBsAg may be undetected both in early infection and late after infection. Specimens containing precipitate may give inconsistent test results.
2. As the 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 entire content of wells completely before adding the washing solution.
3. The positive control in the test kit is not to be used to quantify assay sensitivity. The positive control is used to verify that the test kit components are capable of detecting a reactive specimen provided the procedure is followed as defined in the kit and the storage conditions have been strictly adhered to.











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	<i>In vitro</i> 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