An enzyme immunoassay (EIA) for the qualitative detection of Hepatitis B Surface Antigen (HBsAg) in human serum or plasma.

For professional in vitro diagnostic use only.

INTENDED USE

The HbsAg EIA Test Kit is a one step enzyme immunoassay for the qualitative detection of Hepatitis B Surface Antigen (HBsAg) in human serum or plasma.

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 through sexual contact, blood borne exposure, transmission from infected mother to newborn or via contaminated needles or other sharp objects. The appearance of HBsAg indicates that the body has a significant load of virus and that active infection is taking place. HBsAg is produced by infected liver cells and is released into the bloodstream. The presence of HBsAg in the blood indicates the possibility of infecting others.

The HbsAg EIA Test Kit is a third generation immunoassay for the qualitative detection of the presence of Hepatitis B Surface Antigen in human serum or plasma. The test utilizes monoclonal antibodies to selectively detect various subtypes of HBsAg in serum or plasma.

Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not pipette by mouth.

Avoid any contact of the Substrate A, Substrate B, and Stop Solution with skin or mucosa. The Stop Solution should be handled with care. Do not store Stop Solution in a shallow dish or return it to the original bottle after use.

Materials Required But Not Provided

Graduated cylinders for wash buffer dilution
Calibrated microplate reader capable of reading at 450 nm
Timer
Calibrated automatic or manual microwell plate washer capable of washing 384 wells in 400 mL with a 670-700 nm reference filter, or reading at 450 nm without a reference filter
Disposable gloves
Automated processor (optional)

PRECAUTIONS

Allow reagents and specimens to reach room temperature (15-30°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits. Controls are provided to aid the user in evaluating the performance of the assay system. Any deviation from the procedural requirements may invalidate the results.

Avoid cross contamination between reagents to ensure valid test results. Follow the wash procedure to ensure optimum assay performance. Use Plate Sealer to cover microwell plate during incubation to minimize evaporation. Use a pipet tip for each specimen assayed. Do not mix reagents from other kits with different lot numbers. Avoid cross contamination during wash steps to ensure valid test results.

Follow the wash procedure to ensure optimum assay performance. Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.

Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with fingertips.

Avoid cross contamination during wash steps to ensure valid test results.

No reagent or specimen contact with the skin or eyes. To avoid cross contamination, drop the conjugate on the bottom of the well using a micropipette.

Avoid any cross contamination between reagents to ensure valid test results.

Materials Provided

HBsAg Microwell Plate 1 plate (96 wells/plate)
HBsAg Conjugate 1 x 5 mL
Concentrated Wash Buffer (20x) 1 x 40 mL
Substrate A 1 x 8 mL
Substrate B 1 x 8 mL
Stop Solution 0.5M sulfuric acid 1 x 8 mL
Normal serum negative for HBsAg, HCV, HIV-1, and HIV-2 1 x 1 mL
Inactivated serum containing HBsAg and HCV, HIV-1, and HIV-2 5 x 1 mL
Protein A Sepharose 10mL
Protein G Sepharose 10 mL
Protein A and G columns 10 mL
Sterile Syringe 1 mL 1 x 100 mL
Sterile Filter 1 ML 1 x 100 mL
Nuviril® Solution for decontamination 1 x 100 mL
Nuviril® spray bottle 1 x 100 mL
Graduated cylinders for wash buffer dilution 1 x 10 mL, 5 x 10 mL
Calibrated microplate reader capable of reading at 450 nm with a 670-700 nm reference filter
Calibrated automatic or manual microwell plate washer capable of washing 384 wells in 400 mL with a 670-700 nm reference filter
Calibrated microplate reader capable of reading at 450 nm without a reference filter
Automated processor (optional)

PROCEDURE

Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:25. For the contents of the bottle containing the concentrated wash buffer in a graduated cylinder and fill it with freshly distilled or deionized water to prepare the working wash buffer. The Working Wash Buffer is stable for 2 weeks at 15-30°C.

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Leach A1 as Blank well. Remove unused strips from the microwell plate, and store in the original resealable plate at 4°C. Place microwell plate coated with Anti-HBsAg 1 plate (96 wells/plate) into a Water Bath at 37°C for 1 h. The microwell plate is coated with monoclonal antibodies specific to various subtypes of HBsAg. During testing, the specimen and the enzyme-conjugated HBsAg antibodies are added to the antibody coated microwell plate and then incubated. If the specimen contains HBsAg, it will bind to the antibodies coated on the microwell plate and simultaneously bind the enzyme-conjugated HBsAg. If the specimen does not contain HBsAg, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound HBsAg. Then the enzyme-conjugated HBsAg is incubated with peroxidase, which catalyzes the color change from blue to yellow. The color intensity, which corresponds to the amount of HBsAg in the specimen, is measured with a spectrophotometer at 450 nm.

For professional in vitro diagnostic use only. Do not use after expiration date.

Do not mix reagents from other kits with different lot numbers. Avoid cross contamination during wash steps to ensure valid test results.

Follow the wash procedure to ensure optimum assay performance. Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.

Use a new pipet tip for each specimen assayed.

Ensure that the bottom of the plate is clean and that no bubbles are present on the surface of the liquid before reading the plate. Do not allow wells to dry out during the assay procedure.

Do not touch the bottom of the wells with pipette tips. Do not touch the bottom of the microwell plate with fingertips.

Do not allow sodium hydroxide fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the color reaction may be inhibited.

All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.

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HEALTH AND SAFETY INFORMATION

Some components of this kit contain human blood derivatives. No known test method can offer complete assurance that products derived from human blood may not transmit infrequent agents. Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents be used only by trained personnel in a laboratory setting.

Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.

Mix gently by swirling the microwell plate on a flat bench or other surface.

Cover the microwell plate with the Plate Sealer and store it on a warm plate or in an incubator using one of the following procedures:

Standard Incubation: Incubate at 37°C ± 2°C for 60 min, or at room temperature overnight.

Enhanced Procedure: Incubate at 37°C ± 2°C for 2 min, or at room temperature overnight.

Remove the Plate Sealer. Wash each microwell plate with 350 mL of Working Wash Buffer per well, then remove the liquid.

Turn the microwell plate upside down on absorbent paper to dry and allow it to complete the drying process. Ensure that no liquid is left in the wells.

ProClin™ Solution for disinfection 1 x 100 mL
Cleaning wipes 1 x 100 mL
Nuviril® Solution for decontamination 1 x 100 mL
Nuviril® spray bottle 1 x 100 mL
Graduated cylinders for wash buffer dilution 1 x 10 mL, 5 x 10 mL
Calibrated microplate reader capable of reading at 450 nm with a 670-700 nm reference filter
Calibrated automatic or manual microwell plate washer capable of washing 384 wells in 400 mL with a 670-700 nm reference filter
Calibrated microplate reader capable of reading at 450 nm without a reference filter
Automated processor (optional)
Add 50 μL of Substrate A to each well. (Clear Reagent)

Add 50 μL of Substrate B to each well.

Mix evenly and cover microwell plate with Plate Sealer and incubate in a water bath or incubator using one of the following procedures:
- Standard Procedure: Incubate at 37°C ± 2°C for 10 minutes 1 minute.
- Enhanced Procedure: Incubate at 37°C ± 2°C for 30 minutes ± 2 minutes.

Remove the Plate Sealer.

Add 60 μL of Stop Solution to each well. (Clear Reagent)

Read at 450/630-700 nm within 30 minutes. Note: Microwell plate can also be read at 450 nm, but it is strongly recommended to read it at 450±630-700 nm for better results.

1. The HBsAg EIA Test Kit is used for the detection of HBsAg in human serum or plasma. Diagnosis using the HBsAg EIA Test Kit has been determined using reference HBsAg standards, ad as subtypes. The analytical sensitivity of the HBsAg EIA Test Kit is >99.9%, and the clinical specificity is 99.9%.

2. Check the validation requirements below to determine if the test results are valid.

3. Calculate the Cut-Off Value using the following formula if the test results are valid.

4. The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

5. Calculate the Mean Absorbance of Negative Control and Positive Control by referring to the table below.

6. The test results are considered reactive for HBsAg if the Mean Absorbance after subtraction of Blank Absorbance is above 0.100.

7. NOTE: Specimens with values within ±10% of the Cut-Off Value should be retested in duplicates for final interpretation.

**LIMITATIONS**

1. The HBsAg EIA Test Kit is used for the detection of HBsAg in human serum or plasma. Diagnosis of an infectious disease should not be established based on a single test result. Further testing, including confirmatory testing, should be performed before a specimen is considered positive. A non-reactive test result does not exclude the possibility of exposure. Specimens containing precipitate may give inconsistent test results. Mutated HBsAg may not be detectable by the test.

2. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.

3. As with other sensitive immunoassays, there is the possibility that non-repeatable reactive results may occur due to inadequate washing. The results may be affected due to procedural or instrument error.

4. False positive results may occur due to high titers of Heterophilic Anti Mouse Antibodies (HAMA). Erroneous result may also be due to fibrin particles and microbial contamination.

5. False negative results may occur if the quantity of HBsAg present in the specimen is lower than the analytical sensitivity of the test, or if HBsAg is not present during the stage of the disease when the specimen was collected.

6. 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 speci

**INTERPRETATION OF RESULTS**

**NON-REACTIVE:** Specimens with absorbance less than the Cut-Off Value are non-reactive for HBsAg and may be considered negative.

**REACTIVE:** Specimens with absorbance greater than or equal to the Cut-Off Value are considered initially reactive for HBsAg. The specimen should be retested in duplicate before final interpretation. Specimens that are reactive in at least one of the re-tests are presumed to be reactive and should be confirmed using other HBV markers or confirmatory testing. Specimens that are non-reactive on both re-tests should be considered non-reactive.

**NOTE:** Specimens with values within ±10% of the Cut-Off Value should be retested in duplicates for final interpretation.

**VALIDATION REQUIREMENTS AND QUALITY CONTROL**

1. Calculate the Mean Absorbance of Negative Control and Positive Control by referring to the table below.

2. Check the validation requirements below to determine if the test results are valid.

3. The test results are considered invalid if the above validation requirements are not met. Repeat the test or contact your local distributor.

4. Calculate the Cut-Off Value using the following formula if the test results are valid.

5. The test results are considered reactive for HBsAg if the Mean Absorbance after subtraction of Blank Absorbance is above 0.100.

6. NOTE: Specimens with values within ±10% of the Cut-Off Value should be retested in duplicates for final interpretation.