# HBsAg EIA Test Kit
# Package Insert

| Code: E-102196 | English |
|---|---|

*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. It is intended for screening and as an aid in the diagnosis of possible Hepatitis B infection.

## SUMMARY

HBsAg is one of the earliest markers that appear in the blood following infection with Hepatitis B virus (HBV).[1] This infection of the liver is transmitted through sexual contact, blood borne exposure, transmission from mother to child during delivery, sharing of objects that pierce the skin, child-to-child and household contact. The four major HBsAg subtypes include adw, adr, ayw, and ayr, all sharing the common determinant 'a'. The HBV infection causes a wide variety of liver damage such as acute self-limiting infection, fulminating hepatitis, chronic hepatitis with progression to cirrhosis and liver failure, and asymptomatic chronic carrier state. In 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). HBsAg appears 1-10 weeks after exposure and before biochemical evidence of liver disease or jaundice.[2,3] Three weeks after the onset of acute hepatitis almost half of the patients will still be positive for HBsAg. In the chronic carrier state, HBsAg persists for 6-12 months with no seroconversion to the corresponding antibodies. Therefore, screening for HBsAg is highly recommended for all donors, pregnant women and people in high-risk groups.
The HBsAg EIA Test Kit is a third generation immunoassay for the qualitative detection of the presence of Hepatitis B Surface Antigen in serum or plasma specimen. The test utilizes monoclonal antibodies to selectively detect various subtypes of HBsAg in serum or plasma.

## PRINCIPLE

The HBsAg EIA Test Kit is a solid phase qualitative enzyme immunoassay based on a sandwich principle for the detection of HBsAg in human serum or plasma. 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 to the conjugate to form immobilized antibody-HBsAg-conjugate complexes. If the specimen does not contain HBsAg, the complexes will not be formed. After initial incubation, the microwell plate is washed to remove unbound materials. Substrate A and substrate B are added and then incubated to produce a blue color, indicating the amount of HBsAg present in the specimen. Sulfuric acid solution is added to the microwell plate to stop the reaction which produces a color change from blue to yellow. The color intensity, which corresponds to the amount of HBsAg present in the specimen, is measured with a microplate reader at 450/630-700 nm or 450 nm.

## PRECAUTIONS

- 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 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 new pipet tip for each specimen assayed.
- Ensure that the bottom of the plate is clean and dry 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 hypochlorite 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.

## 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 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.
- 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.
- ProClin™ 300 is included as a preservative in the Conjugate, Concentrated Wash Buffer, Substrate and Controls. Avoid any contact with skin or eyes.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not mouth pipette.
- Avoid any contact of the Substrate A, Substrate B, and Stop Solution with skin or mucosa. The Stop Solution contains 2M sulfuric acid which is a strong acid. If spills occur, wipe immediately with large amounts of water. If the acid contacts the skin or eyes, flush with large amounts of water and seek medical attention.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- Handle and dispose all specimens and materials used to perform the test as if they contained infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.
- Observe Good Laboratory Practices when handling chemicals and potentially infectious material. Discard all contaminated material, specimens and reagents of human origin after proper decontamination and by following local, state and federal regulations.
- Neutralized acids and other liquids should be decontaminated by adding sufficient volume of sodium hypochlorite to obtain a final concentration of at least 1.0%. A 30 minute exposure to a 1.0% sodium hypochlorite may be necessary to ensure effective decontamination.

## STORAGE AND STABILITY

- Unopened test kits should be stored at 2-8°C upon receipt. All unopened reagents are stable through the expiration date printed on the box if stored between 2-8°C. Once opened, all reagents are stable for up to 3 months after the first opening date if stored between 2-8°C. Return reagents to 2-8°C immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch and remove the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch with desiccant supplied at 2-8°C and can be used within 3 months of the opening date. Return the remaining unused strips and supplied desiccant to the original resealable pouch, firmly press the seal closure to seal the pouch completely and immediately store at 2-8°C.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.
- Do not store Stop Solution in a shallow dish or return it to the original bottle after use.

## SPECIMEN COLLECTION AND PREPARATION

- The HBsAg EIA Test Kit can be performed using only human serum or plasma collected from venipuncture whole blood.
- EDTA, sodium heparin, and ACD collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not use specimens with fibrin particles or contaminated with microbial growth.
- Do not leave specimens at room temperature for prolonged periods. Serum and plasma specimens may be stored at 2-8°C for up to 7 days prior to assaying. For long term storage, specimens should be kept frozen below -20°C.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

## REAGENTS AND COMPONENTS

### Materials Provided

| No. | Reagent | Component Description | Quantity | | |
|---|---|---|---|---|---|
| | | | 96 wells/kit | 480 wells/kit | 48 wells/kit |
| | HBsAg Microwell Plate | Microwell plate coated with Anti-HBsAg | 1 plate (96 wells/plate) | 5 plates (96 wells/plate) | 1 plate (48 wells/plate) |
| 1 | HBsAg Conjugate | Anti-HBsAg bound to peroxidase; Preservative: 0.1% ProClin™ 300 | 1 x 8 mL | 5 x 8 mL | 1 x 4 mL |
| 2 | Concentrated Wash Buffer (25x) | Tris-HCl buffer containing 0.1% Tween 20; Preservative: 0.1% ProClin™ 300 | 1 x 40 mL | 5 x 40 mL | 1 x 20 mL |
| 3 | Substrate A | Citrate-phosphate buffer containing hydrogen peroxide; Preservative: 0.1% ProClin™ 300 | 1 x 8 mL | 5 x 8 mL | 1 x 4 mL |
| 4 | Substrate B | Buffer containing tetramethylbenzidine (TMB); Preservative: 0.1% ProClin™ 300 | 1 x 8 mL | 5 x 8 mL | 1 x 8 mL |
| 5 | Stop Solution | 2M Sulfuric acid | 1 x 8 mL | 5 x 8 mL | 1 x 4 mL |
| 6 | HBsAg Negative Control | Normal serum non-reactive for HBsAg, HCV, HIV-1, and HIV-2; Preservative: 0.1% ProClin™ 300 | 1 x 1 mL | 5 x 1 mL | 1 x 0.5 mL |
| 7 | HBsAg Positive Control | Inactivated serum containing HBsAg and negative for HCV, HIV-1, and HIV-2; Preservative: 0.1% ProClin™ 300 | 1 x 1 mL | 5 x 1 mL | 1 x 0.5 mL |
| | Plate Sealers | | 2 | 10 | 2 |
| | Package Insert | | 1 | 1 | 1 |

### Materials Required But Not Provided

- Freshly distilled or deionized water
- Sodium hypochlorite solution for decontamination
- Absorbent paper or paper towel
- Water bath or incubator capable of maintaining 37°C ± 2°C
- Calibrated automatic or manual microwell plate washer capable of aspirating and dispensing 350 µL/well
- Disposable gloves
- Calibrated micropipettes with disposable tips capable of dispensing 50 and 100 µL
- Graduated cylinders for wash buffer dilution
- Vortex mixer for specimen mixing (optional)
- Timer
- Disposable reagent reservoirs
- Calibrated microplate reader capable of reading at 450 nm with a 630-700 nm reference filter, or reading at 450 nm without a reference filter
- Automated processor (optional)

## DIRECTIONS FOR USE

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. Arrange the controls so that well A1 is the Blank well. From well A1, arrange the controls in a horizontal or vertical configuration. The procedure below assigns specific wells arranged in a vertical configuration. Configuration may depend upon software.

| Step | Detailed Procedure | Simplified Procedure |
|---|---|---|
| 0 | • Leave A1 as Blank well. | • Leave A1 as Blank well |
| 1 | • Add 100 µL of Negative Control in wells B1 and C1. (Blue Reagent)<br>• Add 100 µL of Positive Control in wells D1 and E1. (Red Reagent)<br>• Add 100 µL of specimen to assigned wells starting at F1.<br>• Remove unused strips from the microwell plate, and store in the original resealable pouch at 2-8°C. | • B1 and C1: Add 100 µL Negative Control<br>• D1 and E1: Add 100 µL Positive Control<br>• Starting F1: Add 100 µL specimen<br>• Remove and store unused strips at 2-8°C |
| 2 | • Add 50 µL of Conjugate to each well except for the Blank well. (Red Reagent) | • Add 50 µL of Conjugate to each well except for the Blank well |
| 3 | • Mix gently by swirling the microwell plate on a flat bench for 30 seconds.<br>• Cover the microwell plate with the Plate Sealer and incubate in a water bath or an incubator using one of the following procedures:<br>  • Standard Procedure: Incubate at 37°C ± 2°C for 60 minutes ± 2 minutes.<br>  • Enhanced Procedure: Incubate at 37°C ± 2°C for 120 minutes ± 2 minutes. | • Mix gently<br>• Cover the microwell plate with the Plate Sealer and incubate using one of the following procedures:<br>  • Standard Procedure: Incubate at 37°C for 60 min<br>  • Enhanced Procedure: Incubate at 37°C for 120 min |
| 4 | • Remove the Plate Sealer.<br>• Wash each well 5 times with 350 µL of Working Wash Buffer per well, then remove the liquid.<br>• Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried<br>Note: Improper washing may cause false positive results. | • Remove the Plate Sealer<br>• Wash each well 5 times with 350 µL of Working Wash Buffer<br>• Turn the microwell plate upside down on absorbent tissue |
| 5 | • Add 50 µL of Substrate A to each well. (Clear Reagent)<br>• Add 50 µL of Substrate B to each well. (Clear Reagent)<br>Then a blue color should develop in wells containing Positive specimens. | • Add 50 µL of Substrate A to each well<br>• Add 50 µL of Substrate B to each well |
| 6 | • Mix gently then cover microwell plate with Plate Sealer and incubate in a water bath or incubator using one of the following procedures:<br>  • Standard Procedure: Incubate at 37°C ± 2°C for 10 minutes ± 1 minute.<br>  • Enhanced Procedure: Incubate at 37°C ± 2°C for 30 minutes ± 2 minutes. | • Mix then cover microwell plate with Plate Sealer and incubate using one of the following procedures:<br>  • Standard Procedure: Incubate at 37°C for 10 min<br>  • Enhanced Procedure: Incubate at 37°C for 30 min |
| 7 | • Remove the Plate Sealer.<br>• Add 50 µL of Stop Solution to each well. (Clear Reagent)<br>Then a yellow color should develop in wells containing Positive specimens. | • Remove the Plate Sealer<br>• Add 50 µL of Stop Solution to each well |
| 8 | • 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. | • Read at 450/630-700 nm within 30 min |

## AUTOMATED PROCESSING

Automatic EIA microplate processors may be used to perform the assay after validating the results to ensure they are equivalent to those obtained using the manual method for the same specimens. Incubation times may vary depending on the processors used but do not program less incubation times than the procedure listed above. When automatic EIA microplate processors are used, periodic validation is recommended to ensure proper results.

## VALIDATION REQUIREMENTS AND QUALITY CONTROL

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

**Example of Negative Control Calculation**

| Item | Absorbance |
|---|---|
| Negative Control: Well B1 | 0.023 |
| Negative Control: Well C1 | 0.021 |
| Total Absorbance of Negative Control | 0.023 + 0.021 = 0.044 |
| Mean Absorbance of Negative Control | 0.044/2 = 0.022 |
| Blank Absorbance: Well A1 | 0.002 |
| NCx: Mean Absorbance of Negative Control – Blank Absorbance | 0.022 - 0.002 = 0.020 |

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

| Item | Validation Requirements |
|---|---|
| Blank Well | Blank Absorbance should be < 0.050 if read at 450/630-700 nm<br>Note: It should be < 0.100 if read at 450 nm |
| Negative Control | Mean Absorbance after subtraction of Blank Absorbance should be < 0.100 |
| Positive Control | Mean Absorbance after subtraction of Blank Absorbance should be > 1.000 |

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

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

**Example of Cut-Off Value Calculation**

| Item | Absorbance |
|---|---|
| NCx | 0.020 |
| Cut-Off Value: NCx + 0.070 | 0.020 + 0.070 = 0.090 |

## 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-test are presumed to be repeatedly reactive and should be confirmed using other HBV markers or confirmatory testing. Specimens that are non-reactive on both retests should be considered non-reactive.

***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 detected 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 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 specimen provided the procedure is followed as defined in the kit and the storage conditions have been strictly adhered to.

## PERFORMANCE CHARACTERISTICS

### Analytical Sensitivity

The analytical sensitivity of the HBsAg EIA Test Kit has been determined using reference HBsAg standards, ad and ay subtypes. The analytical sensitivity is 0.2 IU/mL using the standard procedure and 0.1 IU/mL using the enhanced procedure, which were all confirmed using the WHO NISBC International Standard with code number 01/476-011-WIL for HBsAg. The analytical sensitivity is 0.2 ng/mL for the standard procedure and 0.1 ng/mL for the enhanced procedure.

### Clinical Sensitivity and Specificity

The HBsAg EIA Test Kit has correctly identified specimens of a seroconversion panel and has been compared with a leading commercial HBsAg EIA test using clinical specimens. The results show that the clinical sensitivity of the HBsAg EIA Test Kit is >99.9%, and the clinical specificity is 99.9%.

**HBsAg EIA vs. Other EIA**

| Method | | Other EIA | | Total Results |
|---|---|---|---|---|
| | Results | Positive | Negative | |
| HBsAg EIA | Positive | 562 | 3 | 565 |
| | Negative | 0 | 5,234 | 5,234 |
| Total Results | | 562 | 5,237 | 5,799 |

Clinical Sensitivity: >99.9% (99.4-100.0%)* Clinical Specificity: 99.9% (99.8-100.0%)*
Overall Agreement: 99.9% (99.9-100.0%)* *95% Confidence Interval

### Reproducibility

**Intra-Assay:** Within-run precision has been determined by using 10 replicates of three specimens: a low positive, a medium positive, and a high positive.

**Inter-Assay:** Between-run precision has been determined by 3 independent assays on the same three specimens: a low positive, a medium positive, and a high positive. Three different lots of the HBsAg EIA Test Kit have been tested using these specimens over a 5-day period.

| Specimen | Intra-Assay | | | Inter-Assay | | |
|---|---|---|---|---|---|---|
| | Mean Absorbance/ Cut-Off | Standard Deviation | Coefficient of Variation (%) | Mean Absorbance /Cut-Off | Standard Deviation | Coefficient of Variation (%) |
| 1 | 1.467 | 0.008 | 6.061 | 1.367 | 0.011 | 8.943 |
| 2 | 12.622 | 0.060 | 5.282 | 13.378 | 0.091 | 7.558 |
| 3 | 26.722 | 0.096 | 3.992 | 25.467 | 0.225 | 9.817 |

## BIBLIOGRAPHY


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2. Krugman, S. Glies J.P. *Viral Hepatitis, Type B (MS-2-Strain). Further Observations on Natural History and Prevention*. New England Journal of Medicine. 288, 755.
3. Krugman, S. Overby L.R, et al. *Viral Hepatitis Type B Studies On Natural History and Prevention Re-examined*. New England Journal of Medicine. 300, 101.


### Index of Symbols

| | | | | | |
|---|---|---|---|---|---|
| | Consult instructions for use | Σ | Tests per kit | | Manufacturer |
| IVD | For *in vitro* diagnostic use only | | Use by | REF | Catalog # |
| 2°C – 8°C | Store between 2-8°C | LOT | Lot Number | Substrate B | Substrate B |
| HBsAg | HBsAg | Substrate A | Substrate A | Control + | Positive Control |
| Wash Buffer 25x | Wash Buffer (25x) | Conjugate | Conjugate | Package Insert | Package Insert |
| Control – | Negative Control | Plate Sealer | Plate Sealer | | |
| Microwell Plate | Microwell Plate | Stop Solution | Stop Solution | | |


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