# Epitope Diagnostics, Inc.

# *EDI*™ HBsAg EIA KIT

*Enzyme Linked Immunosorbent Assay for the Detection Of Hepatitis B Surface Antigen*

**Catalog Number: KT 1008** **Store at 2 – 8ºC Upon Receipt**

**For research use only** **Not for use in diagnostic procedure**

## INTENDED USE

This EIA is a qualitative enzyme immunoassay for the detection of Hepatitis B surface antigen (HBsAg) in serum or plasma of human or infected animals.

## SUMMARY OF PHYSIOLOGY

Hepatitis B virus (HBV) is a member of the Hepadnavirus family. The virus Cat.icle, (virion) consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein. Hepatitis B virus (HBV) infects the liver of hominoidae, including humans, and causes an inflammation called hepatitis. It is a DNA virus and one of many unrelated viruses that cause viral hepatitis. The hepatitis B virus primarily interferes with the functions of the liver by replicating in liver cells, known as hepatocytes. During HBV infection, the host immune response causes both hepatocellular damage and viral clearance. Although the innate immune response does not play a significant role in these processes, the adaptive immune response, Particularly virus-specific cytotoxic T lymphocytes (CTLs), contributes to most of the liver injury associated with HBV infection. By killing infected cells and by producing antiviral cytokines capable of purging HBV from viable hepatocytes, CTLs eliminate the virus. Although liver damage is initiated and mediated by the CTLs, antigen-nonspecific inflammatory cells can worsen CTL-induced immunopathology, and platelets activated at the site of infection may facilitate the accumulation of CTLs into the liver.

Transmission of hepatitis B virus results from exposure to infectious blood or body fluids containing blood. Possible forms of transmission include (but are not limited to) unprotected sexual contact, blood transfusions, re-use of contaminated needles & syringes, and vertical transmission from mother to child during childbirth. Without intervention, a mother who is positive for the hepatitis B surface antigen confers a 20% risk of passing the infection to her offspring at the time of birth. This risk is as high as 90% if the mother is also positive for the hepatitis B e antigen. HBV can be transmitted between family members within households, possibly by contact of non-intact skin or mucous membrane with secretions or saliva containing HBV.

The tests for detection of hepatitis B virus infection involve serum or blood tests that detect either viral antigens (proteins produced by the virus) or antibodies produced by the host. Interpretation of these assays is complex and can be summarized as follows.

The hepatitis B surface antigen (*HBsAg*) is most frequently used to screen for the presence of this infection. It is the first detectable viral antigen to appear during infection. However, early in an infection HbsAg may not be present and it may be undetectable later in the infection as it is being cleared by the host. The infectious virion contains an inner "core Cat.icle" enclosing viral genome. The icosahedral core Cat.icle is made of 180 or 240 copies of core protein, alternatively known as hepatitis B core antigen, or *HBcAg*. During this 'window' in which the host remains infected but is successfully clearing the virus, IgM antibodies to the hepatitis B core antigen (*anti-HBc IgM*) may be the only serological evidence of disease.

Shortly after the appearance of the HBsAg, another antigen named as the hepatitis B e antigen (*HBeAg*) will appear. Traditionally, the presence of HBeAg in a host's serum is associated with much higher rates of viral replication and enhanced infectivity; however, variants of the hepatitis B virus do not produce the 'e' antigen, so this rule does not always hold true. During the natural course of an infection, the HBeAg may be cleared, and antibodies to the 'e' antigen (*anti-HBe*) will arise immediately afterwards. This conversion is usually associated with a dramatic decline in viral replication.

If the host is able to recover from the infection, the HBsAg will become undetectable and will be followed by IgG antibodies to the hepatitis B surface antigen and core antigen, (*anti-HBs* and *anti HBc IgG*) (Fig. 1) . A person negative for HBsAg but positive for anti-HBs has either recovered from an infection or has been vaccinated previously (Fig. 2).

Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers. Carriers of the virus may have chronic hepatitis B, which would be reflected by elevated serum alanine aminotransferase levels and inflammation of the liver, as revealed by biopsy. Carriers who have seroconverted to HBeAg negative status, Cat.icularly those who acquired the infection as adults, have very little viral multiplication and hence may be at little risk of long-term complications or of transmitting infection to others.

Furthermore, PCR tests have been developed to detect and measure the amount of viral nucleic acid in clinical specimens. These tests are called viral loads and are used to assess a person's infection status and to monitor treatment.

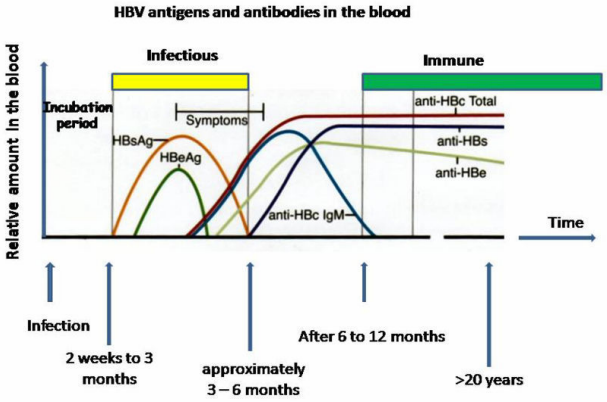


Fig. 1. Hepatitis B viral antigens and antibodies detectable in the blood following acute infection.

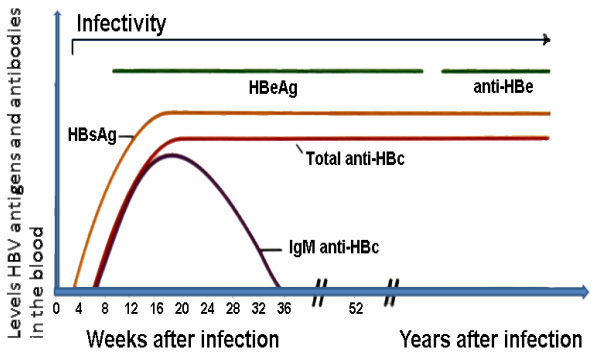


Fig. 2. Hepatitis B viral antigens and antibodies detectable in the blood of a chronically infected person

## ASSAY PRINCIPLE

This HBsAg EIA is a solid-phase sandwich immunoassay by using monoclonal antibodies and polyclonal antibodies specific for HBsAg. Microtiter plate is coated with monoclonal antibodies specific for HBsAg. Assay controls and unknown serum specimens are added to the antibody coated microtiter plate together with enzyme conjugated HBsAg specific polyclonal antibodies. In the presence of HBsAg, a monoclonal antibody-HBsAg-polyclonal antibody-enzyme complex will form. The plate is then washed to remove unbound matrix. Finally, a solution of substrate is added to the wells and incubated. A blue color will develop and the intensity of the color is of proportion to the amount of HBsAg present in the specimen. The enzyme-substrate reaction can be stopped and the result is read by an ELISA plate reader at the wavelength of 450 nm.

## REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

**Prior to use allow all reagents to come to room temperature.**

Regents from different kit lot numbers should not be combined or interchanged.

### Materials provided with the kits

1. **Anti-HBsAg Antibody Coated Plate (Cat. # 30362)**

   Microtiter Plate coated with monoclonal anti-HBs antibody: 96 tests in one pouch. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. **HRP Conjugated Anti-HBsAg Antibody (Cat. # 30363)**

   This is the tracer antibody for the assay. One bottle contains 6.0 ml HRP-conjugated polycloncal anti-HBsAg antibody in a stabilized matrix. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. **HBsAg Negative Control (Cat. # 30364)**

   One vial contains 0.5ml HBsAg Negative Control in a stabilized buffer. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. **HBsAg Positive Control (Cat. # 30365)**

   One vial contains 0.5ml HBsAg Positive Control in a stabilized buffer (Red colored). This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

5. **Wash Buffer Concentrate- 20 x (Cat. # 30275)**

   One bottle contains 20 fold concentrate wash buffer. Before use the contents must be diluted 20 folds with distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiration date on the kit box.

6. **Substrate Solution A (Cat. # 30277)**

   One bottle contains 6 ml HRP Substrate. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

7. **Substrate Solution B (Cat. # 30278)**

   One bottle contains 6 ml TMB Chromogen Substrate. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

8. **Stop Solution (Cat. # 30276)**

   One bottle contains 6 ml 2N Sulfuric Acid. This reagent is ready to use. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

## STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed pouch to minimize exposure to air. Use up the reagents as soon as possible after the kit is unpacked.

## SAFETY PRECAUTIONS

The reagents must be used in research laboratory and are for research use only. Source material from which reagents of bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases. 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. Wear gloves while performing this assay and handle these reagents as if they are potential infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. Upon contact, flush with copious amounts of water for at least 15 minutes. Dispose off all specimens and materials used to perform the test as if they contained infectious agents. Use Good Laboratory Practices.

## MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 25 µL, 50 µL, 100 µL, and 1000 µL.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm glass or plastic tubes.
5. Disposable plastic 1000 mL bottle with caps.
6. Aluminum foil.
7. Plastic microtiter well cover or polyethylene film.
8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
9. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

## SPECIMEN COLLECTION

Either serum or plasma can be used in this test. No special preparation of individual or subject is necessary prior to specimen collection. Whole blood should be collected by venipuncture and must be allowed to clot for a minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg for 10 minutes). The serum should be separated from the clot within two hours of blood collection and transferred to a clean test tube. Grossly hemolytic or lipemic samples should not be used. Serum samples should be stored at 2 - 8ºC if the assay is to be performed within 48 hours. Otherwise, serum samples should be stored at –20ºC or below until measurement. Avoid any repeated freezing and thawing of specimen.

## ASSAY PROCEDURE

**1. Reagent Preparation**

(1) Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.
(2) ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.

**3. Assay Procedure**

(1) Place a sufficient number of anti-HBsAg antibody coated microwell strips in a holder to run assay controls and unknown samples in duplicate.
(2) Test Configuration

| ROW | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| A | Blank | S2 | | | | | |
| B | C 1 | S2 | | | | | |
| C | C 1 | S3 | | | | | |
| D | C 1 | S3 | | | | | |
| E | C 2 | | | | | | |
| F | C 2 | | | | | | |
| G | S 1 | | | | | | |
| H | S 1 | | | | | | |

(3) Dispense one drop (50 µl) of Positive Control as well as Negative Control in duplicate into respective wells. Set one black well as background control.
(4) Pipet 50ul of serum or plasma samples into respective wells
(5) Add one drop (50 µl) of HRP-Conjugated Anti-HBsAg Antibody to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 min. Do not add this conjugated antibody to the blank well.
(6) Place the microtiter plate into a humidified box and incubate at 37°C for 30 min.
(7) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(8) Add one drop (50 µl) of Substrate Solution A to each well, then add one drop (50 µl) of Substrate Solution B to each well. Mix gently and incubate at 37°C for 15 min.
(9) Add one drop (50 µl) of Stop Solution to each well to stop the color reaction. Read O.D. at 450 nm with an EIA reader.

*NOTE: To reduce the background, one can set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 595 nm or 620 nm or 630 nm.*

## PROCEDURAL NOTES

1. It is recommended that all controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light sensitive reagents in the original amber bottles.
3. Store any unused antibody coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading
7. All reagents should be mix gently and thoroughly prior use. Avoid foaming.

## INTERPRETATION OF RESULTS

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):

The OD of positive control must be between 1.4 to 2.8.

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

## LIMITATION OF THE PROCEDURE

1. HBsAg kit is used for the detection of HBsAg in serum or plasma of human or infected animals. Levels of HBsAg may be undetected both in early infection and late after infection.

Specimens containing precipitate may give inconsistent test results.
2. HBsAg EIA is limited to the detection and semi-quantitative of HBsAg in serum or plasma. 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.
4. This test is for research use only and must not be used in diagnostic procedure.

## QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known positive levels of HBsAg. We recommend that all assays include the laboratory's own control samples in addition to those provided with this kit.

## WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

## REFERENCES


1. Magnius L.O., Lindhoim, A. Lundin, P and Iwarson, S.A., New andgen-antibody system. Clinical significance in long-term carriers of hepatitis B surface antigen. .Am.Med.Assoc.231:356-359, 1975.
2. Aldershvile, J. et al., Hepatitis H e antigen and anti-body measured by radioimmunoassay in acute hepads B surface antigen positive hepatitis. J. Infect . Dis. 141: 293-298, 1980.
3. Mushahwar, I.K. et al., Prevalence of hepatitis B e antigen and its antibody as detected by radioimmuncassays. J. Med. Virol. 2: 77-87, 1978.
4. Lin, C.M. et al., Hepatitis B e antigen and its correlation with other serological markers in chimpanzees. Inf. Imm. 24: 352-356, 1979.
5. Lander, JJ., et al. Frequency of antibody to hepatitis associated antigen as measured by a new radioimmunoassay technique. J. Immunol. 106:1166-1171, 1971.
6. Zuckerman AJ (1996). Hepatitis Viruses. In: Baron's Medical Microbiology (Baron S et al, eds.) (4th ed. ed.). Univ of Texas Medical Branch. ISBN 0-9631172-1-1.
7. McMahon BJ (2007). "Chronic hepatitis B". Hepatology 45 (2): 507–39. doi:10.1002/hep.21513.


**TECHNICAL ASSISTANCE AND CUSTOMER SERVICE**
For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678. www.epitopediagnostics.com

This product is developed and manufactured by
**Epitope Diagnostics, Inc.**
San Diego, CA 92126, USA

**Short Assay Procedure:**

1. Add 50 µL of controls and unknown serum/plasma samples into the designated microwell.
2. Add 50 µL of enzyme conjugated antibody into each well.
3. Cover and incubate the plate at 37°C for 30 minutes.
4. Wash each well 5 times.
5. Add 50 µL of Substrate Solution A and 50 µL of Substrate Solution B into each of the wells.
6. Cover and incubate plate at 37°C for 15 minutes.
7. Add 50 µL of ELISA Stop Solution into each of the wells.
8. Read the absorbance at 450 nm.

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If the host is able to recover from the infection, the HBsAg will become undetectable and will be followed by IgG antibodies to the hepatitis B surface antigen and core antigen, (*anti-HBs* and *anti HBc IgG*) (Fig. 1) . A person negative for HBsAg but positive for anti-HBs has either recovered from an infection or has been vaccinated previously (Fig. 2).

Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers. Carriers of the virus may have chronic hepatitis B, which would be reflected by elevated serum alanine aminotransferase levels and inflammation of the liver, as revealed by biopsy. Carriers who have seroconverted to HBeAg negative status, Cat.icularly those who acquired the infection as adults, have very little viral multiplication and hence may be at little risk of long-term complications or of transmitting infection to others.

Furthermore, PCR tests have been developed to detect and measure the amount of viral nucleic acid in clinical specimens. These tests are called viral loads and are used to assess a person's infection status and to monitor treatment.

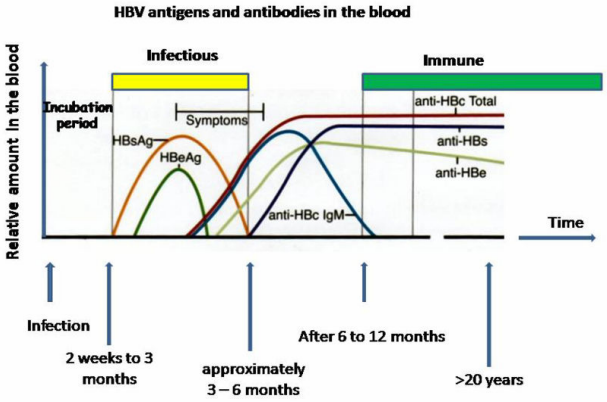


Fig. 1. Hepatitis B viral antigens and antibodies detectable in the blood following acute infection.

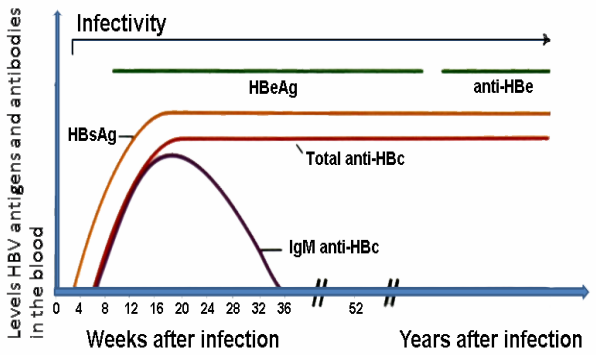


Fig. 2. Hepatitis B viral antigens and antibodies detectable in the blood of a chronically infected person

## ASSAY PRINCIPLE

This HBsAg EIA is a solid-phase sandwich immunoassay by using monoclonal antibodies and polyclonal antibodies specific for HBsAg. Microtiter plate is coated with monoclonal antibodies specific for HBsAg. Assay controls and unknown serum specimens are added to the antibody coated microtiter plate together with enzyme conjugated HBsAg specific polyclonal antibodies. In the presence of HBsAg, a monoclonal antibody-HBsAg-polyclonal antibody-enzyme complex will form. The plate is then washed to remove unbound matrix. Finally, a solution of substrate is added to the wells and incubated. A blue color will develop and the intensity of the color is of proportion to the amount of HBsAg present in the specimen. The enzyme-substrate reaction can be stopped and the result is read by an ELISA plate reader at the wavelength of 450 nm.

## REAGENTS: Preparation and Storage

This test kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

**Prior to use allow all reagents to come to room temperature.**

Regents from different kit lot numbers should not be combined or interchanged.

### Materials provided with the kits

1. **Anti-HBsAg Antibody Coated Plate (Cat. # 30362)**

   Microtiter Plate coated with monoclonal anti-HBs antibody: 96 tests in one pouch. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

2. **HRP Conjugated Anti-HBsAg Antibody (Cat. # 30363)**

   This is the tracer antibody for the assay. One bottle contains 6.0 ml HRP-conjugated polycloncal anti-HBsAg antibody in a stabilized matrix. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

3. **HBsAg Negative Control (Cat. # 30364)**

   One vial contains 0.5ml HBsAg Negative Control in a stabilized buffer. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

4. **HBsAg Positive Control (Cat. # 30365)**

   One vial contains 0.5ml HBsAg Positive Control in a stabilized buffer (Red colored). This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

5. **Wash Buffer Concentrate- 20 x (Cat. # 30275)**

   One bottle contains 20 fold concentrate wash buffer. Before use the contents must be diluted 20 folds with distilled water and mixed well. Upon dilution this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide preservative. The diluted wash buffer should be stored at room temperature and is stable until the expiration date on the kit box.

6. **Substrate Solution A (Cat. # 30277)**

   One bottle contains 6 ml HRP Substrate. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

7. **Substrate Solution B (Cat. # 30278)**

   One bottle contains 6 ml TMB Chromogen Substrate. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box.

8. **Stop Solution (Cat. # 30276)**

   One bottle contains 6 ml 2N Sulfuric Acid. This reagent is ready to use. This reagent should be stored at 2 – 8°C or room temperature and is stable until the expiration date on the kit box.

## STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed pouch to minimize exposure to air. Use up the reagents as soon as possible after the kit is unpacked.

## SAFETY PRECAUTIONS

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## MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 25 µL, 50 µL, 100 µL, and 1000 µL.
2. Repeating dispenser suitable for delivering 100 µL.
3. Disposable pipette tips suitable for above volume dispensing.
4. Disposable 12 x 75 mm glass or plastic tubes.
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8. ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
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## SPECIMEN COLLECTION

Either serum or plasma can be used in this test. No special preparation of individual or subject is necessary prior to specimen collection. Whole blood should be collected by venipuncture and must be allowed to clot for a minimum 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg for 10 minutes). The serum should be separated from the clot within two hours of blood collection and transferred to a clean test tube. Grossly hemolytic or lipemic samples should not be used. Serum samples should be stored at 2 - 8ºC if the assay is to be performed within 48 hours. Otherwise, serum samples should be stored at –20ºC or below until measurement. Avoid any repeated freezing and thawing of specimen.

## ASSAY PROCEDURE

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**3. Assay Procedure**

(1) Place a sufficient number of anti-HBsAg antibody coated microwell strips in a holder to run assay controls and unknown samples in duplicate.
(2) Test Configuration

| ROW | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| A | Blank | S2 | | | | | |
| B | C 1 | S2 | | | | | |
| C | C 1 | S3 | | | | | |
| D | C 1 | S3 | | | | | |
| E | C 2 | | | | | | |
| F | C 2 | | | | | | |
| G | S 1 | | | | | | |
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(3) Dispense one drop (50 µl) of Positive Control as well as Negative Control in duplicate into respective wells. Set one black well as background control.
(4) Pipet 50ul of serum or plasma samples into respective wells
(5) Add one drop (50 µl) of HRP-Conjugated Anti-HBsAg Antibody to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 min. Do not add this conjugated antibody to the blank well.
(6) Place the microtiter plate into a humidified box and incubate at 37°C for 30 min.
(7) Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
(8) Add one drop (50 µl) of Substrate Solution A to each well, then add one drop (50 µl) of Substrate Solution B to each well. Mix gently and incubate at 37°C for 15 min.
(9) Add one drop (50 µl) of Stop Solution to each well to stop the color reaction. Read O.D. at 450 nm with an EIA reader.

*NOTE: To reduce the background, one can set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set at 595 nm or 620 nm or 630 nm.*

## PROCEDURAL NOTES

1. It is recommended that all controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light sensitive reagents in the original amber bottles.
3. Store any unused antibody coated strips in the foil Ziploc bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading
7. All reagents should be mix gently and thoroughly prior use. Avoid foaming.

## INTERPRETATION OF RESULTS

EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):

The OD of positive control must be between 1.4 to 2.8.

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

## LIMITATION OF THE PROCEDURE

1. HBsAg kit is used for the detection of HBsAg in serum or plasma of human or infected animals. Levels of HBsAg may be undetected both in early infection and late after infection.

Specimens containing precipitate may give inconsistent test results.

2. HBsAg EIA is limited to the detection and semi-quantitative of HBsAg in serum or plasma. 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.
4. This test is for research use only and must not be used in diagnostic procedure.

## QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known positive levels of HBsAg. We recommend that all assays include the laboratory's own control samples in addition to those provided with this kit.

## WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

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**Short Assay Procedure:**

1. Add 50 µL of controls and unknown serum/plasma samples into the designated microwell.
2. Add 50 µL of enzyme conjugated antibody into each well.
3. Cover and incubate the plate at 37°C for 30 minutes.
4. Wash each well 5 times.
5. Add 50 µL of Substrate Solution A and 50 µL of Substrate Solution B into each of the wells.
6. Cover and incubate plate at 37°C for 15 minutes.
7. Add 50 µL of ELISA Stop Solution into each of the wells.
8. Read the absorbance at 450 nm.