HBsAg ELISA Kit
Catalog E0710

- **96-well ELISA kit for the Qualitative Detection of HBsAg in Human Serum, plasma.**
- **For Export Only, Not for Re-sale In the USA.**
- **Not for Internal or External Use in Humans or Animal.**
- **Store at 2 - 8ºC Upon Receipt.**

INTENDED USE

HBsAg ELISA is used for the qualitative determination of Hepatitis B surface antigen (HBsAg) in human serum or plasma. It is for professional used only.

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.

TEST PRINCIPLE

The HBsAg ELISA kit utilizes pairs of monoclonal antibodies to detect HBsAg qualitatively and selectively in serum or plasma. The test microwell was coated with one monoclonal antibody. The HBsAg, if present in the test sample, react simultaneously with the antibody immobilized on the microwell surface and with another monoclonal antibody conjugated to horseradish peroxidase enzyme (HRP) to form an antibody-antigen-antibody enzyme complex on the microwell surface. Upon addition of TMB substrate, the bound enzyme generates color. The intensity of the color is directly proportional to the concentration of HBsAg in the samples.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

1. 12 x8-well strips coated with monoclonal anti HBsAg ***(Cat#E0710W)***
2. 1 ml *HBsAg* negative control (*Cat#E0710N)*
3. 1 ml *HBsAg* positive control (*Cat#E0710P)*
4. 12 ml Anti HbsAg-HRP conjugates (*Cat#E0710H)*
5. 20 ml Wash Buffer (30xconcentrate) (*Cat#WE3000)*
6. 11 ml TMB substrate (*Cat#TME2000*)
7. 6 ml Stop solution (*Cat#SE1000)*
8. 1 set Product insert
9. 2 sets ELISA Working Sheet

Materials and reagents required but not provided in the kit

1. Pipette capable of delivering 5-50 ul volumes with a precision better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 50ul and 200ul ml volumes with a precision better than 1.5%.
3. Microplate 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.
4. Absorbent paper for blotting the microplate wells.
5. Timer.
6. Distill water or deionized water.

STORAGE AND STABILITY

- Test components are stable up to their expiration data when stored at 2º-8ºC. Do not freeze.
- Return all reagents requiring refrigeration immediately after use.
- Reseal the microwells immediately after removing the desired number of wells.
- Do not mix or use components from the kits with different lot numbers. Do not use reagents after their expiration date.

WARNING AND PRECAUTIONS

The test kit does not contain any viable infectious agents. However all patient samples must be considered as potentially infectious.

- Do not ingest the reagents. Avoid contact with eyes, skin and mucose. Wear protective clothing and disposable gloves. Do not allow smoking or eating where antigen containing materials are being handled.
- Preclude any pipetting by mouth.
- Handle all patient samples and test components as though capable of transmitting infection. Clean spills thoroughly using an appropriate intermediate-to high level disinfectant. Decontaminate and dispose of specimens and all potentially contaminated materials as if they contain infectious agents.
- Avoid splashing or aerosol formation.
- Avoid contacting Substrate Reagent or Stop solution with the skin or other mucose membranes. In case of contact, wash thoroughly with water.

SPECIMEN COLLECTION AND PREPARATION

- Serum should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
- This kit is designed for use with serum sample without additives only.
- If a specimen is not tested immediately, refrigerated at 2º-8ºC. If storage period greater than three days are anticipated, the specimen should be frozen (-20ºC). Avoid repeated freezing-thawing of samples. If a specimen is to be shipped, pack in compliance with federal regulation covering the transportation of etiologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to assaying.
- Do not use serum samples demonstrating gross lipermia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PROCEDURE NOTES

- Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instruction.
- Do not splash liquid while rocking or shaking the wells.
- The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.
- Avoid strong light during color development. In the beginning of each incubation and after adding Stopping Solution, gently rocking the microwells to ensure thorough mixing. Avoid the formation of air bubbles as which results in inaccurate absorbance values.

ASSAY PROCEDURE

A: Preparation

1. Bring all reagents, standards, controls to room temperature (18-25 ºC).
2. Prior to use, prepare a working dilution of Washing Buffer from the 30x concentration provided. Add 580 ml of distill water to 20 ml concentrated Washing Buffer and mix well. Warm up the Washing Buffer to dissolve precipitates if appear
3. Mix each reagent before adding to the test wells.
4. Determine the number of microwells needed and mark on the ELISA data sheet with the appropriate information. Positive, and Negative Controls require be run in duplicate to ensure accuracy. Blank well is not necessary, an optional.

B: Assaying

1. Remove the microwell strips and reseal un-used strips.
2. Dispense 50 µl of negative control, positive control, and test samples into the appropriated well.
3. Add 50 µl of Anti HBsAg-HRP Conjugate solution into each well, but not blank well.
4. Gently rock the wells for twenty second, then cover the wells.

5. Incubate the wells at 37°C for 60 minutes for sensitivity at 0.5ng/ml, or 120 minutes for sensitivity at 0.2 ng/ml.
6. Carefully remove the incubation mixture by empting the solution into a waste container. Fill each well with diluted wash buffer and shake gently for 20-30 second. Discard the wash solution completely by inverting and tapping the plate on absorbent paper. Repeat above procedure 4 more times.
7. Dry the wells by firmly tapping the plate on a clean paper towel to remove excess washing solution.
8. Add 100 ul (2 drops) of TMB substrate into each well.
9. Incubate 37°C in the dark for 15 minutes.
10. Stop the reaction by adding 50 ul of stop buffer to each well. Gently mix for 30 seconds. **It is important to make sure that all the blue color changes to yellow color completely.**
11. Set the microplate reader wavelength at 450 nm and measure the absorbance of each well within 15 minutes after adding Stop Solution. A filter of 620 -690 nm can be used as a reference wavelength to optimize the assay result.

INTERPRETATION OF RESULTS

A: Calculate OD ratio

$$\text{Specimen OD ratio} = \frac{\text{OD Value of test sample}}{\text{Average OD Value of Negative Control}}$$

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.

B. Interpretations

	Specimen OD ratio
Negative	**<** 2.1
Positive	**≥** 2.1

- The negative result indicates that there is no detectable HBsAg in the specimen.
- The specimen with a positive result should be tested duplicate again and confirmed with Western blot or other tests.

C. Quality Control

1. The OD value of negative control should not exceed 0.10.
2. The OD value of positive control should be above o.50.

Check the procedure and repeat assay if above conditions are not met.

LIMITATION OF THE TEST

1. HBsAg kit is used for the detection of HBsAg in human serum of 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.

REFERENCES

1. Rubin. E. Acute and chronic viral hepatitis. Federation Proceedings. 38 (13): 2665-2673 (1979).
2. Aach R.D., Grisham J.W., Paker C.W. Detection of Australia ant igen by radioimmunoassay. Proc. Natl. Acad. Sci. USA68:1056 (1971).
3. Kim. C.Y. and Tikes. J.G. Purification and biophysical characterization of hepatitis antigen. J. Clin Incest. 52: 1176-1186 (1973) .
4. Ling C. M. Radioimmunoassay for hepatitis B virus. Manual of clinical immunology. Rose N. and Firiedman H. editors.
5. Caldwell. C.W., Barrel.J.T. Enzyme immunoassay for hepatitis B and its comparison to other methods. Clin chem Acta. 81:305 (1977).
6. Wolters G. et al., Enzyme linked immunoasorbent essay for hepatitis B surface antigen. J. Infect. Dis. 136:311 (1977)
7. Magnius L.O., Lindhoilil, A Lunchn, P and Carson. S.A. New antigen-antibody system. Clinical significance in long-term, carriers of hepatitis B surface antigen. J. Am. Med. Asso. 231:356-359 (1975).
8. Pderson, D.L.. Nath N and Gravilanes E Structure of hepatitis B surface antigen. The J d Bid.Chern. 257 (17):10414-10410 (1982).