

Hepatitis B Surface Antigen (HBsAg) ELISA Kit

For the Detection of Hepatitis B Surface Antigen
(HBsAg)
in Human Plasma or Serum.

Catalogue Number: EL10018

96 tests

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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TABLE OF CONTENTS

	Page
INTENDED USE	2
INTRODUCTION	2
PRINCIPLE OF THE ASSAY	2
REAGENTS PROVIDED	3
MATERIALS REQUIRED BUT NOT SUPPLIED	4
PRECAUTIONS	4
SAMPLE PREPARATION	5
.....Collection, Handling, and Storage	5
PREPARATION OF REAGENTS	5
ASSAY PROCEDURE	6
CALCULATION & INTERPRETATION OF RESULTS	7-8

INTENDED USE

This HBsAg ELISA Kit is to be used for the *in vitro* detection of Hepatitis B surface antigen (HBsAg) including the subclass of HBsAg (ad, ay.) in human plasma or serum. This kit is intended for RESEARCH USE ONLY, and is not to be used in diagnostic or therapeutic procedures.

INTRODUCTION

Hepatitis B is an infection of the liver caused by the hepatitis B virus. Approximately 5-10% of adults and 90% of babies who are infected with HBV will go on to carry the virus for the rest of their lives. These people will pass the virus onto others. HBV is excreted in body fluids such as semen, saliva, blood and urine in persons with acute or chronic infection. The route of transmission can include homosexual or heterosexual activity, blood-borne exposure (needles, transfusion), mother-infant, close personal contact and even by consuming contaminated food or water. Thus, Hepatitis B has become a major public health concern.

When HBV invades the body it causes liver damage through induction of auto-immunity. Liver cell injury results from cytotoxic T cell activity rather than viral cytotoxic activity. The principle screening test for detecting current (acute or chronic) HBV infection is the identification of HBsAg, an envelope lipoprotein. This is the first immunological marker, to appear in a patient's serum and exist in high quantities in the blood. Patients who are HBsAg positive develop chronic persistent hepatitis (CPH) and chronic active hepatitis (CAH). Patients with CPH usually remain in good health but those with CAH have progressive liver damage with the outcome being portal fibrosis, cirrhosis, or hepatocellular carcinoma.

Screening for HBsAg is recommended for all donors, pregnant women and those individuals who at high risk. Presence of this marker is conclusive proof of HBV infection. YBL offers an immunoassay that can measure HBsAg through antigen-antibody interactions.

PRINCIPLE OF THE ASSAY

Monoclonal antibodies specific for HBsAg have been bound to the surface of each microplate well. During the course of the assay, the positive control, negative control and samples are added to the microplate wells. The microtiter plate wells are thoroughly washed to remove unbound other components of the sample. . A standardized preparation of horseradish peroxidase (HRP) conjugated antibody specific for HBsAg is added to each well to "sandwich" the antibodies immobilized during the first incubation. Following a wash to remove any unbound HRP conjugate, a TMB (3,3', 5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a 10-minute incubation period. The enzyme-substrate reaction is terminated by the addition of sulfuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm±2nm. Only those wells containing HBsAg and HRP conjugate will exhibit a change in color. The intensity of this color change is proportional to the concentration of HBsAg in the sample.

REAGENTS PROVIDED

All reagents provided are stored at 4°C. Refer to the expiration date on the label.

	96 tests
1. MICROTITER PLATE (Part EL18-1)	96 wells
Pre-coated with murine anti-human HBsAg monoclonal antibody.	
2. CONJUGATE (Part EL18-2)	15 mL
Horseradish peroxidase (HRP) conjugated anti-human HBsAg antibody. Ready to use.	
3. NON-REACTIVE CONTROL (NRC) (Part EL18-3)	0.5 mL
Inactivated normal human serum.	
4. REACTIVE CONTROL (RC) (Part EL3-4)	0.5 mL
Inactivated HBsAg positive human serum diluted in buffered solution with animal protein.	
5. WASH BUFFER (20X) (Part EL18-5)	60 mL
20-fold concentrated solution of buffered surfactant.	
6. SUBSTRATE A (Part EL18-6)	10 mL
Buffered solution with H ₂ O ₂ .	
7. SUBSTRATE B (Part EL18-7)	10 mL
Buffered solution with TMB.	
8. STOP SOLUTION (Part EL18-8)	11 mL
2N sulphuric acid solution (H ₂ SO ₄). Caution: Caustic Material!	

MATERIALS REQUIRED BUT NOT PROVIDED

1. Single or multi-channel pipettes with disposable tips: 5-100 μL and 50-200 μL for running the assay.
2. Pipettes: 1 mL, 5 mL, 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L, and 2 L.
7. Incubator ($37\pm 2^\circ\text{C}$)
8. Microtiter plate reader ($450\text{nm}\pm 2\text{nm}$).
9. Automatic microtiter plate washer or squirt bottle.
10. Sodium hypochlorite solution, 5.25% (household liquid bleach).
11. Deionized or distilled water.
12. Plastic plate cover
13. Disposable gloves.
14. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Controls, conjugate, and microtiter plates are matched for optimal performance. Use only reagents supplied by manufacturer.
2. Allow kit reagents to reach room temperature ($20\text{-}25^\circ\text{C}$) before use. Do not use water baths to thaw reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from storage bag until needed. Unused strips should be stored at $2\text{-}8^\circ\text{C}$ in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum, plasma and the Controls in the Kit should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since 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 and good laboratory practices should be followed.
9. All materials should be disposed of in a manner that will inactivate human viruses.
Solid Waste: Autoclave 60 min. at 121°C .
Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone, keep reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature ($2\text{-}5^\circ\text{C}$), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

COLLECTION, HANDLING, AND STORAGE

- a) **Serum:** Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted.
- b) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection there must be quick separation of plasma with less than 30 minutes on ice. Centrifuge for 10 minutes (4°C) to remove any particulate. *This HBsAg ELISA Kit is not affected by haemolysis of specimens. No adverse effects have been noted in the presence of anti-coagulants, sodium citrate, EDTA, or heparin.*
 - Avoid grossly hemolytic, lipidic or turbid samples
 - Serum or plasma samples to be used within 24-48 hours may be stored at 2-8°C otherwise samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
 - When performing the assay slowly bring samples to room temperature.
 - It is recommended that all samples be assayed in duplicate.
 - DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below.

1. **Non-Reactive Control and Reactive Control:** Supplied in prediluted form. DO NOT DILUTE.
2. **Wash Buffer:** Dilute 1 volume of Wash Buffer (20X) with 19 volumes of distilled or deionized water. For example dilute 60 mL of wash buffer (20X) into deionized or distilled water to prepare 1200 mL of wash buffer (1X). Mix thoroughly by gently swirling before use. Avoid foaming. Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.
2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Wells Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
16 wells	1.5	1.5	3.0
32 wells	3.0	3.0	6.0
48 wells	4.0	4.0	8.0
64 wells	5.0	5.0	10.0
80 wells	6.0	6.0	12.0
96 wells	7.0	7.0	14.0

ASSAY PROCEDURE

1. Prepare Wash Buffer before starting assay procedure (see Preparation of Reagents). *It is recommended that the table provided be used as a reference for adding Controls and Samples to the Microtiter Plate.*

Wells	Contents	Wells	Contents
A1, B1	Substrate Blank	E1, F1	Reactive Control (RC)
C1, D1	Non-Reactive Control (NRC)	G1.....	Samples

2. Secure the required number of coated strips in the microplate holder.
3. Pipette 100 μ L of each control (Non-Reactive Control, and Reactive Control) and sample to the appropriate Microtiter Plate well. ***Remember to leave 2 wells empty for Substrate Blank.*** Cover and incubate for 30 minutes at 37°C.

4. Wash the Microtiter Plate using one of the specified methods indicated below:

Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note:* Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

Automated Washing: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer. Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μ L/well/wash (range: 350-400 μ L). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears.

5. Add 100 μ L of Conjugate into each well. Cover and incubate for 30 minutes at 37°C. ***Remember to leave 2 wells empty for Substrate Blank.***
6. Prepare Substrate Solution (see Preparation of Reagents) no more than 15 minutes before end of incubation.
7. Repeat wash procedure as described in Step 4.
8. Add 100 μ L of Substrate Solution to each well, ***including those reserved for substrate blank.*** Cover and incubate for 10 minutes at 37°C.
9. Add 50 μ L Stop Solution to each well, ***including those reserved for substrate blank.*** Mix well.
10. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes. *Use Substrate Blank as microtiter plate reader blank.*

EXAMPLE 2: Sample B (O.D. value) = 1.895
 NRC (mean O.D.) = 0.055

$$\frac{1.895}{0.055} > 2.1 \quad \text{Sample B is positive}$$

EXAMPLE 3: Sample C (O.D. value) = 0.116
 NRC (mean O.D.) = 0.055

$$\frac{0.105}{0.055} = 2.1 \quad \text{Sample C is positive}$$