HBV surface antigen / HBsAg ELISA Kit

Enzyme Immunoassay kit for the determination of HBsAg in serum and plasma (EDTA, heparin, citrate).

Catalog number: ARG82305

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION **Page**

INTRODUCTION 3
PRINCIPLE OF THE ASSAY 3
MATERIALS PROVIDED & STORAGE INFORMATION 4
MATERIALS REQUIRED BUT NOT PROVIDED 4
TECHNICAL HINTS AND PRECAUTIONS 5
SAMPLE COLLECTION & STORAGE INFORMATION 6
REAGENT PREPARATION 7
ASSAY PROCEDURE 7
CALCULATION OF RESULTS 8
VALIDITY OF THE TEST 10
QUALITY ASSURANCE 10

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INTRODUCTION

HBsAg (also known as the Australia antigen) is the surface antigen of the hepatitis B virus (HBV). It indicates current hepatitis B infection.

Today, these antigen-proteins can be genetically manufactured (e.g. transgene E. coli) to produce material for a simple antigen test, which detects the presence of HBV. It is present in the sera of patients with viral hepatitis B (with or without clinical symptoms). Patients who developed antibodies against HBsAg (anti-HBsAg seroconversion) are usually considered non-infectious. HBsAg detection by immunoassay is used in blood screening, to establish a diagnosis of hepatitis B infection in the clinical setting (in combination with other disease markers) and to monitor antiviral treatment. [Provide by Wikipedia: HBsAg]

PRINCIPLE OF THE ASSAY

This assay employs the sandwich enzyme immunoassay technique. A capture antibody specific for HBsAg has been pre-coated onto a microtiter plate. Controls or samples are pipetted into the wells and any HBsAg present is bound by the immobilized antibody. After washing away any unbound substances, an antibody-conjugate specific for HBsAg is added to each well and incubate. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of HBsAg bound in the initial step. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm. The HBsAg in the sample is then determined by comparing the O.D of samples to the controls.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated microplate	8 X 12 strips	4°C
Positive Control	1.5 ml (Ready-to-use)	4°C
Negative Control	2 ml (Ready-to-use)	4°C
Antibody Conjugate (HRP-conjugated antibody)	8 ml (Ready-to-use)	4°C
20X Wash Buffer	55 ml	4°C
TMB substrate A	12 ml	4°C (Protect from light)
TMB substrate B	12 ml	4°C (Protect from light)
STOP solution	12 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (620 nm as optional reference wave length)
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Deionized or distilled water
- 37°C oven or incubator
- Microtiter plate washer (recommended)
- Fully automatic EIA micro-plate analyzer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C at all times. Microtiter wells must be stored at 2 - 8°C. Once the foil bag has been opened, care should be taken to close it tightly again and used it within 1 month after opening.
- Return reagents to 2-8°C immediately after use. Opened kits retain activity for 1 month if stored as described above.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Take care not to contaminate the TMB Substrate. Do not expose the TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- Do NOT return leftover TMB Substrate to bottle. Do NOT contaminate the unused TMB Substrate. If the solution is blue before use, DO NOT USE IT.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Change pipette tips between the addition of different reagent or samples.
- Plate readers measure vertically. Do not touch the bottom of the wells.
- The positive control, standards, conjugate solution and Samples should be regarded as potential health hazards. It should be used and discarded

according to your own laboratory's safety procedures.

- Potential infectious Samples and nonacid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with the local procedures for potential bio-hazard control.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at -20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

a) This reagent kit is to be used for un-pooled human serum or plasma only.

b) The reagent kit has not been validated for use with cadaveric samples.

c) Do not use haemolytic, icteric or lipaemic Samples.

d) Samples containing sodium azide should not be used in the assay.

e) Incompletely coagulated serum samples and microbial-contaminated Samples should not be used.

f) Frozen Samples must be thoroughly thawed and mixed homogenously before testing.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 20X Wash buffer into distilled or deionized water (Do not use tap water) to yield 1X Wash buffer, mix well. (E.g. 50 ml of 20X Wash buffer + 950 ml of distilled water) The diluted Wash buffer is stable for 1 weeks at 2°C to 8°C.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (20- 30°C) before use, each vial should be mixed thoroughly without foaming prior to use. Controls should be assayed at least in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl** of each **Control or Sample** into appropriate wells. Keep one well empty as blank. (Duplicates for positive and triplicates for negative control is recommended)
3. Add **50 µl** of **Antibody Conjugate** into each well (**except blank well**, do not touch the well wall to prevent contamination). Gently tap the plate to mix well. Cover wells and incubate for **80 minutes at 37°C**.
4. Aspirate each well and wash, repeating the process 5 times for a total **6 washes**. Wash by filling each well with 1× Wash Buffer (**350 µl**) using a squirt bottle, manifold dispenser, or autowasher, keep the Wash Buffer in the wells for 30 sec before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

5. Prior to use, **mix equal volumes of TMB substrate A and TMB substrate B** in a clean container. Add **100 µl** of the **TMB mixture solution** to each well including the blank well. (The TMB Substrate mixture should be used within 30 minutes after mix. The mixture should be kept away from intense light).
6. Cover the plate with a **Black Cover** and incubate for **30 minutes** at **RT in dark.**
7. Add **100 µl** of **Stop Solution** to each well. The color of the solution should change from blue to yellow. Gently tap the plate to ensure thorough mixing.
8. Read the OD with a microplate reader at **450 nm** reading wavelength with **620 nm** reference wavelength immediately. It is recommended read the absorbance **within 30 minutes** after adding the stop solution.

   (Note: The color of the blank should be colorless to light yellowish; otherwise, the test results are invalid. Substrate blank absorbance value must be less than 0.100.)

CALCULATION OF RESULTS

1. Calculation of the NC (mean absorbance of Negative Control).

   Note: mean of Negative Control should be ≤ 0.1 after subtracting the blank, otherwise, the test is invalid.

2. Calculation of the PC (mean absorbance of Positive Control).

   Note: mean of Positive Control should be ≥ 0.6, otherwise, the test is invalid.

3. Calculation of the PC-NC value.

   Note: PC-NC value must be ≥ 0.5, otherwise, the test is invalid.

4. Calculation of the Cutoff Value.

   Cutoff Value = NC + 0.025

5. Calculate the average absorbance values for each set of controls and samples.

6. Interpretation of Results

   A. Samples with absorbance values **LESS** than the **Cutoff Value** are **NON-REACTIVE** and are considered **NEGATIVE for HBsAg.**

   B. Samples with absorbance value **GREATER** than or **EQUAL** to the **Cutoff Value** are considered **INITIALLY REACTIVE**. The original Samples must be retested in duplicate.

      a) If both absorbance values of the retest samples from above are less than the cutoff value, the Samples are considered **NEGATIVE for HBsAg.**

      b) If in the retest values at least one of the two absorbance values is **GREATER** than or **EQUAL** to the **Cutoff Value** then the Samples are considered as **repeated HBsAg positive**. The repeated positive sample shall be confirmed with certain valid confirmatory reagents.

   C. A negative HBsAg result without other evidence should not be used to exclude an HBV infection.

VALIDITY OF THE TEST

NC should be ≤ 0.1, otherwise, the test is invalid.

PC should be ≥ 0.6, otherwise, the test is invalid.

PC-NC value must be ≥ 0.5, otherwise, the test is invalid.

Note: Negative Control: absorbance value must be less than or equal to 0.100 after subtracting the blank.

QUALITY ASSURANCE

Diagnostic Specificity

Total No. of Samples	HBV surface antigen / HBsAg ELISA kit					
	N	Neg	*IR	**RR	Confirmed	False Positive
HBV negative (clinical Samples)	213	211	2	2	0	2
HBV negative (donor Samples)	5501	5479	22	22	0	22
Total	5714	5690	24	24	0	24

*IR: initial reactive **RR: repeat reactive

Diagnostic specificity = 5690/5714 = 99.58%

Diagnostic Sensitivity

The diagnostic sensitivity determined in the European performance evaluations yielded the following results:

Sample	No. of sample	Reactive	Sensitivity
HBsAg positive sera	400	400	100%

Diagnostic specificity = 400/400 = 100%