

Hepatitis B surface antigen ELISA Kit

Catalog Number KA0286

96 assays

Version: 37

Intended for research use only

www.abnova.com



Table of Contents

Introduction	3
Intended Use	
Background	
Principle of the Assay	
General Information	5
Materials Supplied	5
Storage Instruction	
Materials Required but Not Supplied	6
Precautions for Use	7
Assay Protocol	9
Reagent Preparation	9
Sample Preparation	9
Assay Procedure	9
Data Analysis	12
Calculation of Results	
Performance Characteristics	
Resources	15
Troubleshooting	
References	
Plate Layout	16



Introduction

Intended Use

The Hepatitis B surface antigen ELISA kit is an enzyme immunoassay kit for qualitative detection of hepatitis B surface antigen (HBsAg) in human serum or plasma (heparin, citrate or EDTA).

Background

The hepatitis B surface antigen (HBsAg) is the first marker that appears in the blood following infection with hepatitis B virus (HBV) some days or weeks before clinical symptoms manifest. It is a lipoprotein polypeptide which constitutes the external envelope of the HB virus. The detection of HBsAg in human serum or plasma indicates an ongoing HBV infection, either acute or chronic. Testing of additional HBV markers is needed to define the specific disease state. HBsAg assays are used not only to diagnose HBV infections but also to monitor the course of the disease and the efficacy of antiviral therapy.

The Hepatitis B surface antigen ELISA kit is a fast test for the qualitative detection of the presence of HBsAg in serum or plasma (heparin, citrate or EDTA) specimen. The test utilizes monoclonal and polyclonal (anti-guinea pig) antibodies to selectively detect elevated levels of HBsAg in serum or plasma.

Specimens which are non-reactive by Hepatitis B surface antigen ELISA kit are considered negative for HBsAg. Specimens with positive reaction should be retested in duplicate.

In case of a reactive repeat reaction, the specimen should be confirmed for HBsAg reactivity with validated confirmatory reagents. Only confirmed positive specimens are considered to contain HBsAg.

Principle of the Assay

The Hepatitis B surface antigen ELISA Kit is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immuno-sorbent assay) based on the "sandwich principle".

The solid phase of the microtiter plate is made of polystyrene wells coated with mouse monoclonal antibodies specific for HBsAg; whereas guinea pig polyclonal antibody purified by affinity chromatography is used to prepare the anti-HBs• peroxidase (horseradish) conjugate in the liquid-phase.

When a serum or plasma specimen containing HBsAg is added to the anti-HBs antibody-coated wells together with the peroxidase conjugated anti-HBs antibody and incubated, an antibody-HBsAg-antibody-peroxidase complex will form on the wells.

After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. A color develops in proportion to the amount of HBsAg bound to Anti-HBs. The peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450 nm with a selected reference wavelength within 620 nm to 690 nm^{*1}.



- A. Specimen containing HBsAg:
- 1. Plate well (Anti-HBs) + specimen (HBsAg)+ Anti-HBs•peroxidase→ Anti-HBs•HBsAg•(Anti-HBs•peroxidase) sandwich complex
- 2. Sandwich complex + TMB substrate solution \rightarrow Light blue to blue color
- 3. Add sulfuric acid to stop the color development \rightarrow Read OD at 450 nm (reference wavelength 620-690 nm^{*1})
- B. Specimen without HBsAg:
- 1. Plate well (Anti-HBs) + specimen (no HBsAg) + Anti-HBs•peroxidase → Anti-HBs (on the well)
- 2. Anti-HBs (on the well) + TMB substrate solution \rightarrow Colorless to light blue color
- Add sulfuric acid to stop the color development →Read OD at 450 nm (reference wavelength 620-690 nm*¹)

Note: ^{*1} The reference wavelength of the photometer to be used can be 620 nm to 690 nm. However, the user should validate the photometer in combination with Hepatitis B surface antigen ELISA Kit before use.



General Information

Materials Supplied

List of component

Component	Description	Amount		
Anti-HBs Plate	Microtiter plate coated with mouse monoclonal anti-HBs.	1 plate		
Anti-HBs • Peroxidase Solution	ion Polyclonal Anti-HBs • HRPO conjugate, diluted in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal. Dye: phenol red.			
HBsAg Positive Control	Inactivated human serum positive for HBsAg but non-reactive for anti-HCV and anti-HIV1/2, diluted in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1.5 mL		
Serum non-reactive for HBV markers, anti-HCV and anti-HIV1/2, diluted in buffer with protein stabilizers.Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.		2.0 mL		
TMB Substrate Solution A	3,3',5,5'-tetramethylbenzidine (TMB) in an organic base.			
TMB Substrate Solution B	Acetic Acid Buffer with Urea Hydrogen Peroxidase.			
Conc. Washing Solution D (20X)	Concentrated Phosphate buffer with Tween-20.			
2 N H ₂ SO ₄	2 N sulfuric acid	12 mL		

Accessories: (provided as needed)

Component	Amount	
Adhesive slips	X1	
Black cover	X1	

Storage Instruction

- ✓ The kit must be stored at 2 to 8°C. Do not freeze.
- ✓ Strips of the plate should be used within 1 month after opening the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and taped tightly.
- ✓ Return reagents to 2 to 8°C immediately after use.
- ✓ Conc. Washing Solution D (20x) can be stored at room temperature to avoid crystallization, because the kits are stored at 2 to 8°C. If the crystal has been precipitated before use, warm up the solution in a 37°C water bath till the crystal is dissolved.



✓ Storage condition and Stability of the kit and components
 Washing Solution D (20X) and 2 N H₂SO₄ can be stored at 2-30°C. The other reagents should be refrigerated at 2-8°C.

Klt/components	Storage temp.	State	Stability
	2 - 8°C	Original	18 months
Repatitis B surface antigen ELISA Kit		Once open	1 month
HBsAg Positive Control	2 - 8°C	Original	18 months
		Once open	1 month
HB Negative Control	2 - 8°C	Original	18 months
		Once open	1 month
Anti-HBs Plate	2 - 8°C	Original	24 months
		Once open	1 month
Anti-HBs • Peroxidase Solution	2 - 8°C	Original	18 months
		Once open	1 month
Conc. Washing Solution D (20X)	Room temp.	Original	24 months
		Once open	1 month
20X Diluted Washing Solution	Room temp.	Diluted	2 days
	2 - 8°C	Diluted	1 week
TMD Substrate Solution A	2 - 8°C	Original	24 months
TWD Substrate Solution A		Once open	1 month
TMP Substrate Solution P	2 - 8°C	Original	24 months
		Once open	1 month
TMB Substrate mixture	Room temp.	Mixture	6 hours
	Boom tomp	Original	24 months
2 10 112004		Once open	1 month

Materials Required but Not Supplied

- \checkmark 50 µL, 100 µL micropipettes and tips are needed
- \checkmark Water bath or incubator with temperature control at 37°C.
- Plate washing equipment.
- ✓ ELISA Microwell Reader: Dual wavelength 450 nm with 620-690 nm as reference wavelength, bandwidth 10 nm^{*1}.
- ✓ Fully automatic EIA micro-plate analyzer is optional. User should validate the automatic EIA micro-plate analyzer in combination with the kit.

Note: ^{*1} The reference wavelength of the photometer to be used can be 620 nm to 690 nm. However, the user should validate the photometer in combination with Hepatitis B surface antigen ELISA Kit before use.



Precautions for Use

- This reagent kit is for professional use only.
- This reagent kit is for research only.
- Bring all kit reagents and samples to room temperature 20-30°C and mix carefully before use.
- Do not use reagent beyond its expiration date.
- Do not interchange reagents between different lots.
- Do not put pipette in mouth.
- Do not smoke or eat in areas where specimens or reagents are handled.
- All kit components and specimens should be regarded as potential health hazards. It should be used and discarded according to your laboratory's safety procedures. Such safety procedures probably include the wearing of protective gloves and avoiding the use of aerosols.
- Potential infectious specimens and non-acid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with your practice for potential bio-hazard control.
- Prior to disposing used specimens and kit reagents as general waste; it should be treated in accordance with the local practice of potential bio-hazardous waste or treated as follows:

Both liquid and solid waste should be autoclaved at 121°C for at least 30 minutes.

Solid waste can also be incinerated.

Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%.

Acidic liquid wastes must be neutralized before treatment with sodium hypochlorite as mentioned above and should stand for 30 minutes to obtain effective disinfection.

- 2 N Sulfuric Acid is an irritant to skin, eyes, respiratory tract and mucous membranes. Avoid contact of the 2 N sulfuric acid with skin and mucous membranes. In case of contact, flush immediately with abundant amounts of water. In case of inhalation, find fresh air and seek medical attention in case of pain.
- TMB substrate solution A contains organic solvent, which is flammable. TMB substrate solution A contains dimethyl sulfoxide, an irritant to skin and mucous membranes.
- Although all human sourced material are tested non-reactive for Anti-HCV and Anti-HIV, and inactivated at 56°C for one hour, the reagent shall be handled as potential infectious material*².

Note: ^{*2} Incomplete inactivation of hepatitis B virus after heat treatment at +60°C for 10 hours, J. Infect. Dis. 138:242-244.

- ✓ Limitations and Interferences
- This reagent kit is to be used for un-pooled human serum or plasma only.
- A negative HBsAg result without other evidence should not be used to exclude an HBV infection.
- Interfering Substances:

The following results were obtained in respective experiments:

1. No interferences with different anticoagulants such as lithium heparin, EDTA, citrate have been



observed.

- 2. Heat-treated specimens (+60°C, 10 hours) exhibited diminished HBsAg titer.
- No cross reactivity was detected using specimens deriving from persons with a) other infections by HAV, EBV, CMV, HSV, VZV, Lyme Borreliosis, HCV, HIV, b) other disease states such as chronic renal failure, hemodialysis, autoimmune hepatitis, liver cirrhosis, and c) presence of certain antibodies like HAMA, GAD, IA2, APS).
- 4. Samples containing potential interfering substances [e.g. triglycerides (lipemia), hemoglobin (hemolysis), bilirubin (icterus), monoclonal immunoglobulin components, elevated levels of autoimmune antibodies (rheumatoid factor-RF, antinuclear antibodies-ANA, or antimitochondrial antibodies-ANA)] and samples from pregnant women did not interfere with this assay.



Assay Protocol

Reagent Preparation

- Preparation of washing solution:
 Dilute Conc. Washing Solution D (20X) with distilled or de-ionized water to obtain a 1:20 dilution. Do not use tap water.
- Plate washing:
- 1. a. For plate washer with overflow aspirating function: 6 cycles with at least 0.5 mL washing buffer per well per cycle.

or

b. For plate washer without overflow aspirating function: 8 cycles with at least 0.35 mL washing buffer per well per cycle.

2. Blot dry by inverting the plate and tapping firmly onto absorbent paper. Too much residual wash buffer will cause false results.

Note: Improper washing can cause false result.

Sample Preparation

- ✓ Specimen Collection and Preparation for Analysis
- No special preparation of the person is required prior to blood collection. Blood should be collected by approved techniques.
- Either serum or plasma specimens can be used with this test kit. Whole blood specimen should be separated as soon as possible in order to avoid hemolysis. Any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen should be removed prior to use.
- Specimens must be stored at 2 to 8°C and avoid heat-inactivation to minimize deterioration. For long-term storage, they should be frozen below -20°C. Storage in self-defrosting freezer is not recommended.
- Frozen specimens must be thoroughly thawed and mixed homogenously before test.
- Avoid multiple freeze-thaw procedures.

Note:

- 1. The specimen must not contain any compounds of AZIDE, which inhibits the peroxidase activity.
- 2. Incompletely coagulated sera and microbial-contaminated specimens should not be used.

Assay Procedure

- 1. Bring all reagents and specimens to room temperature (20 to 30°C) before assay. Adjust water bath or incubator to 37±1°C.
- 2. Reserve one well for blank. Add 50 µL of each control or specimen to appropriate wells of the microtiter



plate (3 Negative Controls and 2 Positive Controls).

Note:

- a. Use a clean pipette tip for each sampling to avoid cross-contamination.
- b. Each plate needs respective negative controls, positive controls and blank well.
- c. Do not use any cut-off value established for other plates of the Hepatitis B surface antigen ELISA Kit.
- Add 50 µL of Anti-HBs•Peroxidase Solution to each well except the blank.
 Note: Do not touch the wall of the plate wells to prevent contamination.
- 4. Gently tap the plate.
- 5. Remove the protective backing from the Adhesive Slip and press it onto the reaction plate, so that it is tightly sealed.
- 6. Incubate the reaction plate in 37±1°C water bath or incubator for 80 minutes.
- 7. At the end of the incubation period, remove and discard the Adhesive Slip and wash the plate by "Plate Washing Procedure".
- 8. Select one of the following two methods for color development:
 - ✓ Mix equal volumes of TMB Substrate Solution A and B in a clean container immediately prior to use.
 Add 100 µL of the mixture solution to each well including the blank well.
 - ✓ Add 50 µL of TMB Substrate Solution A first, and then add 50 µL of TMB Substrate Solution B into each well including the blank. Mix well carefully.

Note: TMB Substrate Solution A should be colorless to light blue; otherwise, it should be discarded. The mixture of TMB Substrate Solution A and B should be used within 6 hours after mix. The mixture should be kept away from intense light.

- 9. Cover the plate with a black cover and incubate at room temperature for 30 minutes.
- 10. Stop the reaction by adding 100 μ L of 2 N H₂SO₄ to each well including the blank.
- Determine the absorbance of Controls and test specimens within 30 minutes with a precision spectrophotometer at 450/620-690 nm (450 nm reading wavelength with 620-690 nm reference wavelength)^{*2}.

Use the blank well to blank spectrophotometer

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



✓ Flow chart of the test procedure



Note: ^{*1} The reference wavelength of the photometer to be used can be 620 nm to 690 nm. However, the user should validate the photometer in combination with Hepatitis B surface antigen ELISA Kit before use.



Data Analysis

Calculation of Results

✓	Calculation of the NC (Mean Absorbance of Negative Control)
	Example: Sample No.	Absorbance
	1	0.022
	2	0.025
	3	0.023
	NC = (0.022 + 0.025 +	0.023) / 3 = 0.023
	NC should be \leq 0.1, oth	nerwise, the test is invalid.
✓	Calculation of the PC (I	Mean Absorbance of Positive Control)
	Example: Sample No.	Absorbance
	1	1.432
	2	1.508
	PC = (1.432 + 1.508) /	2 = 1.470
	PC should be \geq 0.6, oth	nerwise, the test is invalid.
√	Calculation of the P - N	Value
	P - N = PC - NC	
	Example: NC = 0.024	
	PC = 1.470	
	P - N = 1.470 - 0.024 =	1.446
	P - N Value must be ≥ 0	0.5, otherwise, the test is invalid.
✓	Calculation of the Cuto	ff Value
	Cutoff Value = NC + 0.0	025
	Example:	
	Cutoff Value = 0.023 +	0.025 = 0.048
√	Validity of Test Runs	
	NC should be \leq 0.1; oth	nerwise, the test Is invalid.
	PC should be ≥ 0.6 , oth	nerwise, the test is invalid.
	P - N Value must be ≥0	0.5, otherwise, the test Is invalid.
	NOTE: Negative Conti	rol: absorbance value must be less than or equal to 0.100 after subtracting the
	blank.	
✓	Interpretation of Result	S
	1. Specimens with	absorbance values LESS than the Cutoff Value are NON-REACTIVE and are
	considered NEGA	TIVE for HBsAg.
	2. Specimens with a	absorbance value GREATER than or EQUAL to the Cutoff Value are considered

- INITIALLY REACTIVE. The original specimens must be retested in duplicate.
- 3. If both absorbance values in the retest are less than the cutoff value, the specimens are considered



NEGATIVE for HBsAg.

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

Performance Characteristics

✓ Analytical Sensitivity

Standard Material	Calculated Sensitivity
WHO 2 nd international Standard*	0.03 IU/mL
PEI standard Subtype ad	0.08 PEI U/mL
PEI standard Subtype ay	0.09 PEI U/mL

*WHO Second International Standards for HBsAg, subtype adw2, genotype A, NIBSC code: 00/588

- ✓ Precision
 - Intra-assay reproducibility

Intra-assay precision was determined using one positive control sample and two person serum samples of different HBsAg concentration (slightly above the cutoff level and at medium level) which were analyzed in replicates of 20 in a single run over 3 days.

Run	Sample ID	Mean	Standard Deviation Coefficient of Variat	
1	PC 1:32	0.7541	0.0857	11.36
1	PS 1:32	2.2766	0.1571	6.90
1	PS 1:64	1.3159	0.1168	8.88
2	PC 1:32	0.9671	0.0358	3.70
2	PS 1:32	2.7325	0.1025	3.75
2	PS 1:64	1.5822	0.0888	5.61
3	PC 1:32	0.9669	0.0909	9.40
3	PS 1:32	2.6088	0.2367	9.07
3	PS 1:64	1.6502	0.1499	9.08

The calculated CV's ranged between 3.7% and 11.36%. (= acceptable value for an immunoassay in microtiter plate format).

Inter-assay reproducibility

The precision evaluation experiments were performed over 10 operating days is using five serum samples (with borderline positive and clearly above cutoff value HBsAg levels).

Sample ID	N	Mean	Standard Deviation	Coefficient of Variation	
F1	10	0.0609	0.0185	30.41	
F2	10	0.0770	0.0189	24.56	
F3	10	0.1000	0.0245	24.51	
F4	10	0.1786	0.0462	25.87	
F5	10	0.6107	0.1412	23.12	



The calculated CV's ranges between 30.4 for an HBsAg negative sample and 23.1 % for an HBsAg low positive sample (= acceptable values for the inter-assay imprecision of an immunoassay in microtiter plate format).

✓ Antigen Excess/High-dose hook effect

This was performed testing a serum sample with a very high HBsAg concentration of 125 mg/L in serial dilution with the Hepatitis B surface antigen ELISA Kit. No high-dose hook effect was observed.

- ✓ Mutants
 - 1. Mutant Panel

A Hepatitis B Pre Core Mutant panel of Teragenix, USA was tested. Hepatitis B surface antigen ELISA Kit can detect the results while panel's dilution ratio is 1:2 or 1:5.

This panel consists of 3 samples Core Promoter (A1762/G1764) wild type; PreCore codon 28 mutant/wild type infection, of 4 samples Core Promotor (A1762/G1764) wild type; PreCore codon 28 mutant, of 2 samples Core Promotor (T1762/A1754) MUTANT/(A1762/G1754) wild type mixed infection.

2. Mutant samples

The detection rate of Hepatitis B surface antigen ELISA Kit is 17/21 which is better than reference kit's (14/21).

Mutation	KA0286	Reference kit	Mutation	KA0286	Reference kit
	Int	Int		Int	Int
113A114	positive	positive	C137W	positive	negative
P120L	positive	positive	C139Y	positive	positive
R122I	negative	negative	K141E	positive	positive
R122T	positive	positive	P142L/G145R	negative	negative
E122I	positive	positive	P142L	positive	positive
T123N	positive	positive	D144G	positive	negative
122RA123	negative	negative	G145K	positive	negative
C124R	negative	negative	C147T/C148T	positive	positive
T131I	positive	positive	E164D	positive	positive
T131P	positive	positive	S174N	positive	positive
Y134S	positive	positive		positive	positive
Detection Rate	8/11	8/11	Detection Rate	9/10	6/10



Resources

Troubleshooting

If the result cannot be reproduced, perform a preliminary troubleshooting by checking the possibilities listed below:

- ✓ Improper washing procedure.
- ✓ Contamination with positive specimen.
- ✓ Wrong volume of sample, conjugate or substrates.
- \checkmark Contamination of the well rim with conjugate.
- Improper specimen, such as hemolyzed serum or plasma, specimen containing sediments and specimen not thoroughly mixed before use.
- ✓ Wrong incubation time or temperature.
- ✓ Obstructed or partial obstructed washer aspirate/dispense head and needles.
- ✓ Insufficient aspiration.

References

- 1. Blumberg BS, Alter HJ, Visnich S. A "new" antigen in leukemia sera. JAMA, 1965;191:101-106.
- 2. Dane DS, Cameron CH, Briggs M. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. Lancet 1970; 1: 695 698.
- 3. Aach RD, Grisham JW, Paker CW. Detection of Australia antigen by radioimmunoassay. Proc Natl Acad Sci. USA 1971;68:1056-1060.
- Kim CY, Tikes JG. Purification and biophysical characterization of hepatitis antigen. J Clin Invest. 1973; 52:1176- 1186.
- Wolters G. Kuijpers LP, Kacaki J, Schuurs AH, Enzyme linked immunosorbent assay for hepatitis B surface antigen. J Infect. Dis 1977;136:Suppl 311-377.
- Shih JW, Cote PJ Jr, Dapolito GM, Gerin JL. Production of monoclonal antibodies against hepatitis B surface antigen (HBsAg)by somatic cell hybrids. J Virol Methods. 1980;1:257-273.
- Hoofnagle JH, Di Bisceglie AM. Serologic diagnosis of acute and chronic viral hepatitis. Semin Liver Dis. 1991;11:73-83.



Plate Layout

12								
11								
10								
თ								
ω								
2								
e								
2								
4								
m								
5								
~								
	A	В	C	Δ	ш	ш	IJ	Т