RecombiLISA

HBsAg ELISA

IVD | REF E0710

- 96-well ELISA kit for the qualitative detection of HBsAg in human serum or plasma
- For export only, not for re-sale in the USA
- Store at 2-8°C upon receipt

INTENDED USE

The *RecombiLISA* HBsAg ELISA is a solid-phase enzyme-linked immunosorbent assay for the qualitative detection of hepatitis B surface antigen (HBsAg) at a sensitivity level of 0.10 IU/mL in human serum or plasma. It is intended for professional use only as an aid in the diagnosis of infection with hepatitis B virus (HBV).

INTRODUCTION

Hepatitis B virus is transmitted through contact with infected blood of other body fluids. It attacks the liver, causing both acute and chronic disease. HBV infects more than 300 million people worldwide and is a common cause of liver disease and liver cancer[1]. In 2015, 887,000 deaths occurred as a result of HBV, primarily from cirrhosis of the liver[2]. It is a particular burden in many developing countries, due to the high cost of prevention, management, and treatment [3,4].

HBV is a small DNA virus that is a member of the Hepadnaviridae family. The core of the virus contains DNA polymerase, as well as core (HBcAg) and envelope (HBeAg) antigens[3,5]. Encompassing the HBV core is a lipid, protein, and carbohydrate coating, which expresses a surface antigen known as HBsAg[3].

HBsAg is one of the first antigen markers detected in the blood[4]. HBsAg may be detected as early as 1–2 weeks after exposure and is persistent for the duration of clinical symptoms. Long term presence (> 6 months) of HBsAg is a marker of chronic HBV infection, and is the primary risk factor for development of chronic liver disease and cancer[4]. Antibody to HBsAg (HBsAb) can be detected at late stages of infection, during recovery after clearance of HBsAg. The presence of HBsAb is usually associated with immunity against HBV[4].

Therefore, HBsAg detection can be used to diagnose both acute and chronic HBV infection, and in combination with other HBV markers such as core and envelope proteins and antibodies, can aid in accurate diagnosis of disease state as well as post-vaccination immunity[6]. HBsAg screening is highly recommended for all blood donors, pregnant women, and people in high-risk groups[6].

TEST PRINCIPLE

The *RecombiLISA* HBsAg ELISA is a solid-phase enzyme-linked immunosorbent assay based on the principle of antibody sandwich technique for the detection of HBsAg in human serum or plasma.

The *RecombiLISA* HBsAg ELISA Test is composed of two key components:
1) Solid microwells pre-coated with monoclonal anti-HBsAg;
2) Liquid conjugate composed of polyclonal HBsAg antibody conjugated with horseradish peroxidase (HRP-HBsAg Antibody Conjugate).

During the assay, the test specimen and HRP-HBsAg Antibody Conjugate are incubated simultaneously in the anti-HBsAg antibody coated microwells. If present, the HBsAg will bind to the anti-HBsAg antibody, as well as the HRP-HBsAg Antibody Conjugate, forming a sandwich antibody complex.

Any unbounded antigen or conjugate is then removed through washing. TMB substrate is then added to the microwells, and the presence of the conjugate complex is shown by development of a blue color resulting from a reaction between the enzyme and substrate. This reaction is then quenched upon addition of the Stop Solution, and the absorbance value for each microwell is determined using a spectrophotometer at 450/620-690 nm.

MATERIALS AND REAGENTS

Materials and reagents required but not provided in the kit

1. Pipettes capable of delivering 50 µL, and 100 µL volume
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-2.5 or greater at 450 nm wavelength is acceptable
3. Absorbent paper for blotting the microwells
4. Timer
5. Distilled or de-ionized water

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	Anti-HBsAg Coated Microwells	8 wells x 12 strips	E0710W
2	HBsAg Negative Control	1 mL	E0710N
3	HBsAg Positive Control	1 mL	E0710P
4	HRP-HBsAg Antibody Conjugate	6 mL	E0710H
5	Wash Buffer (30x Concentrate)	20 mL	WE3000
6	TMB Substrate	12 mL	TME2002
7	Stop Solution	13 mL	SE1002
8	ELISA Working Sheet	2	E0001ES
9	Product Insert	1	PI-E0710
Others	2 x Microplate Sealers and 1 x Resealable Bag		

STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Store all components at 2-8°C. Do not freeze. Avoid strong light. Ensure that the reagents are brought to room temperature before opening. Reseal the microwells after removing the desired number of wells. Place unused wells in the resealable plastic bag provided and return to 2-8°C. Once opened, the kit is stable for 8 weeks at 2-8°C, or until the labeled expiration date, whichever is earlier.

SPECIMEN COLLECTION AND PREPARATION

- Serum or plasma should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
- This kit is designed for use with serum or plasma specimen without additives only.
- If a specimen is not tested immediately, refrigerated at 2-8°C. If storage period greater than three days are anticipated, the specimen should be frozen (-20°C). Avoid repeated freezing-thawing of specimens. 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 specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PREPARATION OF THE REAGENTS

1. Bring all reagents, controls to room temperature (18-28°C).
2. **Preparation of working Wash Buffer:**
   If precipitants are visible, warm up the Wash Buffer (30X concentrate) at 37°C. Dilute concentrated Wash Buffer 30 fold with water as follows:

Plate	DI water	Wash buffer (30X)	Final volume
1 strip	58 mL	2 mL	60 mL
2 strips	116 mL	4 mL	120 mL
3 strips	174 mL	6 mL	180 mL
4 strips	232 mL	8 mL	240 mL

3. Mix each reagent before adding to the test wells.
4. Determine the number of microwells needed and mark on the ELISA Working Sheet with the appropriate information. Positive and Negative Controls should be run in duplicate to ensure accuracy.

ASSAY PROCEDURE

1. Calculate the desired number of microwells. Remove the remaining microwells and place them with desiccant into the resealable plastic bag, seal and store at 2-8°C for later use.
2. Add specimens according to the designation on the ELISA Working Sheet:

2.1 Blank Well: Do not add any reagents.

2.2 Control Wells: Add 50 µL of HBsAg Positive, Negative Control into the designated control wells, respectively.

2.3 Test wells: Add 50 µL of test specimens into each test well, respectively.

3. Add 50 µL of the HRP-HBsAg Antibody Conjugate to each well, except the Blank Well.
4. Gently mix the wells for 20 seconds, and cover the plate with a sealer.
5. Incubate the microwells at 37°C for 90 minutes.
6. Wash Step (Can be performed manually or with automated washing):
   Manual washing: Carefully remove the incubation mixture by disposing the solution into a waste container. Fill each well with 350 µL diluted wash buffer and mix gently for 20-30 seconds. Discard the wash solution completely. Repeat 4 more times. After completing the last wash step, tap the plate on absorbent paper to remove residual liquid.

   Automatic washing: Automatic plate washer must be calibrated to ensure efficient washing. Aspirate incubation mixture from all wells completely. Fill each well with 350 µL diluted wash buffer and soak for 20-30 seconds. Aspirate all wells completely. Repeat 4 more times.
7. Add 100 µL of TMB Substrate into each well, including the Blank Well.
8. Incubate at 37°C in dark for 20 minutes.
9. Stop the reaction by adding 100 µL of Stop Solution to each well. Gently mix for 30 seconds. **It is important to make sure that all the blue color completely changes to a color yellow.**
10. Set the microplate reader wavelength at 450nm. Measure the absorbance (OD) of each well against the Blank 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.

Flow chart of assay procedure

1.	Secure strips in microwell frame	Number of strips
2.	Add controls or specimens, respectively	50 µL
3.	Add HRP-HBsAg Antibody Conjugate (except Blank Well)	50 µL
4.	Gently mix	20 seconds
5.	Incubate	37°C, 90 minutes
6.	Wash: manual or automatic	5 times
7.	Add TMB Substrate	100 µL
8.	Incubate in dark	37°C, 20 minutes
9.	Add Stop Solution. Gently mix	100 µL, 30 seconds
10.	Read result	450/620-690 nm within 15 minutes

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cut-off value = 0.08 + N
N: Mean OD of the negative control. Use 0.05 for calculation of the cut-off value if less than 0.05.

B. Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the cut-off value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cut-off Value}}$$

C. Assay validation

The mean OD value of the HBsAg positive controls should be ≥ 2.0
The mean OD value of the HBsAg negative controls should be ≤ 0.10
Check the assay procedure including incubation time and temperature and repeat assay if above criteria is not met.

D. Interpretation of the results

	Specimen OD ratio
Negative	< 1.00
Positive	≥ 1.00

1. A negative result indicates that there is no detectable HBsAg in the specimen.
2. Specimens with OD ratio ≥ 1.00 are initially considered to be positive by the

RecombiLISA HBsAg ELISA.

3. Results just below the cut-off value (OD ratio between 0.9 and 1) should be interpreted with caution. It is advisable to retest the corresponding specimens in duplicate when applicable.
   If after retesting the OD ratio of one of the duplicates is equal or greater than 1.0, the initial result is repeatable and the specimen is considered to be positive with the *RecombiLISA* HBsAg ELISA, subject to the limitation of the procedure, described below.
   If after a specimen is re-tested the OD ratio values of the duplicates are less than 1, the initial result is non-repeatable and the specimen should be considered negative with the *RecombiLISA* HBsAg ELISA. Samples that have been retested and determined to be negative, but have an OD ratio between 0.9 and 1 should be considered cautiously and retested with another method.
   Non-repeatable reactions may be caused by:
   - Inadequate microwell washing
   - Contamination of negative specimens by serum or plasma with a high HBsAg concentration
   - Contamination of the TMB Substrate by oxidizing agents (bleach, metal ions, etc.)
   - Contamination of the Stop Solution

PERFORMANCE CHARACTERISTICS

1. Analytical Sensitivity

The WHO 3rd International Standard (IS) for HBsAg (12/226) was diluted by 20 confirmed negative plasma specimens at the concentrations: 0.12, 0.10, 0.08, and 0.06 IU/mL, respectively. The samples were tested by *RecombiLISA* HBsAg ELISA. As shown in the table below, the analytical sensitivity, determined as the concentration with 95% detection for the *RecombiLISA* HBsAg ELISA was determined to be 0.10 IU/mL.

HBsAg (12/226) in plasma specimens (IU/mL)	0.12	0.10	0.08	0.06
Number of positive	20	20	12	0
Number of negative	0	0	8	20
Detection rate %	100%	100%	60%	0%

2. Clinical Performance

A total of 425 specimens were collected from susceptible subjects and tested by *RecombiLISA* HBsAg ELISA and by CLIA. Comparison for all subjects is showed in the following table:

	RecombiLISA HBsAg ELISA		
Ref. HBsAg EIA	**Positive**	**Negative**	**Total**
Positive	137	0	137
Negative	0	288	288
Total	137	288	425

Relative Sensitivity: 100% (95% CI: 97.34%-100%)
Relative Specificity: 100% (95% CI: 98.73%-100%)
Overall Agreement: 100% (95% CI: 99.14%-100%)

3. Precision

a. Intra-assay Precision was determined by testing 16 replicates of 9 specimens (3 negative, 3 weak positive, and 3 medium positive) in a single assay. The mean OD value, standard deviation (SD), and coefficient of variance (CV) from all 3 lots are shown in the following table:

Panel	Runs	OD	SD	CV
Negative	16	0.06	0.01	23%
Weak Positive	16	0.40	0.04	9%
Medium Positive	16	0.87	0.06	7%

b. Inter-assay Precision was determined by testing 9 specimens (3 negative, 3 weak positive, and 3 medium positive) in 16 runs using 3 lots. The mean OD, SD, and CV are shown in the following table:

Panel	Runs	OD	SD	CV
Negative	16	0.06	0.01	25%
Weak Positive	16	0.40	0.04	10%
Medium Positive	16	0.87	0.07	7%

4. Cross-Reactivity

No false positive result from the *RecombiLISA* HBsAg ELISA were observed on 3-10 positive specimens from each of the following disease states or special clinical conditions, respectively:

Dengue	HAV	HCV	HIV
H. pylori	*Treponema pallidum*	ANA	HAMA
RF (up to 8400 IU/mL)			

5. Interference

Common substances (such as pain medication or blood components) may affect the performance of the *RecombiLISA* HBsAg ELISA. Interference was studied by spiking these substances into 3 levels of HBsAg specimens: negative, low positive and medium positive. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the *RecombiLISA* HBsAg ELISA.

List of potentially interfering substances and concentrations tested:

Potential Interferent	High Concentration	Low Concentration
Bilirubin	20 mg/dL	6.7 mg/dL
EDTA	3.4 uM	1.1 uM
Salicylic acid	3.8%	1.3%
Creatinine	442 uM	147 uM
Heparin	3000 U/L	1000 U/L
Glucose	55 mmol/L	18 mmol/L

WARNING AND PRECAUTIONS

For *In Vitro* Diagnostic Use

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not use expired kits.
3. Bring all reagents to room temperature (18-28°C) before use.
4. Do not use the components of any other type of test kit as a substitute for the components in this kit.
5. Do not use serum derived from hemolyzed blood specimens for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mouth. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
10. At the beginning of each incubation and after adding Stop Solution, gently rock the microwells to ensure thorough mixing. Avoid the formation of air bubbles which results in inaccurate absorbance values. Avoid splashing liquid while rocking or shaking the wells.
11. Do not allow the microwells to dry between the end of the washing operation and the reagent distribution.
12. The enzyme substrate reaction is very sensitive to metal ions. Thus, do not allow any metal elements to come into contact with the conjugate or TMB Substrate.
13. The enzyme-substrate is temperature dependent. Ensure that the room temperature for TMB incubation falls between 18-28˚C.
14. The TMB substrate must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The TMB Substrate must be stored in the dark.
15. Use a new dispensing tip for each specimen. Never use the specimen container to distribute conjugate and TMB Substrate.
16. **The wash procedure is critical. Wells must be aspirated completely before adding the Wash buffer or liquid reagents. Automatic washers must be validated with the test kit prior to use. Insufficient washing will result in poor precision and falsely elevated absorbance values.**
17. **Microplate reader must be calibrated per manufacturer's instruction to ensure accurate determination of absorbance. Non-calibrated reader often leads to invalid test results.**
18. Avoid exposure to strong light during color development.

LIMITATION OF THE TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of HBsAg in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *RecombiLISA* HBsAg ELISA is limited to the qualitative detection of HBsAg at a sensitivity level of 0.10 IU/mL in human serum or plasma.
3. A negative result for an individual subject indicates absence of detectable HBsAg. However, a negative test result does not preclude the possibility of exposure to or infection with HBV.
4. A negative result can occur if the quantity of HBsAg present in the specimen is below the detection limits of the assay (below 0.10 IU/mL), or the HBsAg that are detected are not present during the stage of disease in which a specimen is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor (above 8400 IU/mL) may affect expected results.
6. Any use or interpretation of this test's results must also rely on other clinical findings and the professional judgment of health care providers.

STANDARDIZATION

The *RecombiLISA* HBsAg ELISA has been calibrated against WHO 3rd IS for HBsAg (12/226) (HBV genotype B4, HBsAg subtypes ayw1/adw2. The Third IS was calibrated against the 2nd IS for HBsAg (A2, adw2) as well as additional study samples representing different HBV genotypes[7].

REFERENCES

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3. Jooste P, et al. Screening, characterisation and prevention of Hepatitis B virus (HBV) co-infection in HIV-positive children in South Africa. J Clin Virol 2016 (85): 71–74. https://doi.org/10.1016/j.jcv.2016.10.017 PMID: 27838494
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5. Kaplan PM, Greenman RL, Gerin JL, Purcell RH, Robinson WS. DNA polymerase associated with human hepatitis B antigen. J Virol. 1973 12(5): 995-1005.
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7. WHO/BS/2014.2241

Index of Symbols

	See instructions for use		Use by
IVD	For *in vitro* diagnostic use only	CONJ	Conjugate
REF	Catalog #	MICROWELLS	Coated microwells
LOT	Lot number	CONTROL +	Positive control
	Tests per kit	CONTROL -	Negative control
	Do not reuse	WASH BUFF	Wash buffer
	Manufacturer	TMB SUBS	TMB substrate
	Date of manufacture	STOP SOLN	Stop solution
2°C 8°C	Store between 2-8°C		

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