RecombiLISA

HBeAg ELISA

IVD | REF E0720

- 96-well ELISA kit for the qualitative detection of hepatitis B e-antigen (HBeAg) in human serum, plasma
- For export only, not for re-sale in the USA
- Store at 2-8°C upon receipt

INTENDED USE

The *RecombiLISA* HBeAg ELISA is a solid-phase enzyme-linked immunosorbent assay for the qualitative detection of hepatitis B e-antigen (HBeAg) in human serum or plasma. It is intended for professional use only as an aid in the diagnosis of infection with Hepatitis virus B (HBV).

INTRODUCTION

HBV is the most common cause of persistent viremia and the most important cause of chronic liver disease and hepatocellular carcinoma. Clinically apparent HBV infections may have been extant for several millennia. It is estimated that there are 300 million chronic carriers of HBV in the world. The carrier rates vary from as little as 0.3% (Western countries) to 20% (Asia, Africa)[1].

HBV is a hepatotropic DNA virus. The core of the virus contains a DNA polymerase[2], the core antigen (HBcAg)[3] and the e antigen (HBeAg)[4]. The core of HBV is enclosed in a coat that contains lipid, protein and carbohydrate and expresses an antigen terms hepatitis B surface antigen (HBsAg)[3].

HBeAg is seen in the blood before the onset of clinical disease and after the appearance of HBsAg. HBeAg generally disappears within about 2 weeks, while HBsAg is still present. The presence of HBeAg in the serum correlates with a period of intense viral replication and hence, maximal infectivity of the patient[1,4].

TEST PRINCIPLE

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

The *RecombiLISA* HBeAg ELISA is composed of two key components:
1) Solid microwells pre-coated with monoclonal anti-HBe antibody (HBeAb)
2) Liquid conjugates composed of polyclonal HBeAb conjugated to horseradish peroxidase (HRP-HBeAb Conjugate)

During the assay, the test specimen and HRP-HBeAb Conjugate are incubated simultaneously in the coated microwells. The HBeAg, if present in the specimen, binds to both the HBeAb coated on the microwell surface and the HBeAb in the HRP Conjugate, forming an antibody sandwich immunocomplex.

Unbound materials are then removed by washing. After addition of the TMB substrate, the presence of the immunocomplex is shown by the development of a blue color resulting from a reaction between the enzyme and substrate. The reaction is then quenched upon addition of the Stop Solution, and absorbance values are determined using a spectrophotometer at 450/620-690 nm.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	HBeAb Coated Microwells	8 wells x 12 strips	E0720W
2	HBeAg Negative Control	1 mL	E0720N
3	HBeAg Positive Control	1 mL	E0720P
4	HRP–HBeAb Conjugate	6 mL	E0720H
5	Wash Buffer (30 x Concentrate)	20 mL	WE3000
6	TMB Substrate A	6 mL	TME2000A
7	TMB Substrate B	6 mL	TME2000B
8	Stop Solution	6 mL	SE1000
9	ELISA Working Sheet	2	E0001ES
10	Product Insert	1	PI-E0720
Others 3 x Microplate Sealers and 1 x Resealable Bag			

Materials and reagents required but not provided in the kit

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

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 bag provided and return to 2-8°C. All the reagents are stable through the expiration date printed on the label if not opened.

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, refrigerate at 2-8°C. If storage period greater than three days is 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.** Warm up the concentrated Wash Buffer to 37°C to dissolve the precipitant if it appears. Dilute concentrated Wash Buffer 30 fold with water as follows:

Plate	DI water	Wash buffer (30X)	Final volume
1 strip	58 mL	2.0 mL	60 mL
2 strips	116 mL	4.0 mL	120 mL
3 strips	174 mL	6.0 mL	180 mL
4 strips	232 mL	8.0 mL	240 mL

The diluted wash buffer can be stored at 2-8˚C for up to 3 days.

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 HBeAg Positive, Negative Control into the designated control wells, respectively.

2.3 Test Wells: Add 50 µL of test specimen into each test well, respectively.

3. Add 50 µL of HRP-HBeAb Conjugate into each well, except the Blank Well.
4. Gently shake the wells for 20 seconds, then cover the wells.
5. Incubate the wells at 37°C for 30 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 of diluted Wash Buffer and shake 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 of diluted wash buffer and soak for 20-30 seconds. Aspirate all wells completely. Repeat 4 more times.
7. Add 50 µL of TMB Substrate A and 50 µL of TMB Substrate B into each well including the Blank Well.
8. Incubate at 37°C in dark for 10 minutes.
9. Stop the reaction by adding 50 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 yellow color.**
10. Set the microplate reader wavelength at 450 nm. 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 HBeAg Positive, Negative Control and specimens, respectively	50 µL 50 µL
3.	Add HRP-HBeAb Conjugate	
4.	Gently shake	20 seconds
5.	Incubate	37°C, 30 minutes
6.	Wash: manual or automatic	5 times
7.	Add TMB Substrate A and B, respectively	50 µL + 50 µL
8.	Incubate in dark	37°C, 10 minutes
9.	Add Stop Solution. Gently mix	50 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 = N x 2.1
N: Mean OD of the negative control. Use N=0.05 for calculation of the cut-off value if the mean OD is 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 HBeAg positive controls should be ≥ 0.50.
The mean OD value of the HBeAg negative controls should be ≤ 0.10.

Check the 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. The negative result indicates that there is no detectable HBeAg in the specimen.
2. Specimens with OD ratio ≥ 1.00 are initially considered to be positive by the *RecombiLISA* HBeAg ELISA.
3. Results within 10% of the cut-off value should be interpreted with caution (it is advisable to retest in duplicate the corresponding specimens when it is applicable).
   If after retesting the absorbance of one of the duplicates is equal or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the *RecombiLISA* HBeAg ELISA, subject to the limitation of the procedure, described below.
   If after retesting of a specimen, the absorbance value of the 2 duplicates are less than the cut-off value, the initial result is non-repeatable and the specimen is considered to be negative with the *RecombiLISA* HBeAg ELISA.
   Non-repeatable reactions are often caused by:
   - Inadequate microwell washing
   - Contamination of negative specimens by serum or plasma with a high antibody titer
   - Contamination of the substrate solution by oxidizing agents (bleach, metal ions, etc.)
   - Contamination of the Stop Solution

PERFORMANCE CHARACTERISTICS

1. Clinical Performance

A total of 300 specimens were collected from susceptible subjects and tested by the *RecombiLISA* HBeAg ELISA and by a Chinese State Drug Administration (SDA) licensed reference ELISA. The comparison for all subjects is showed in the following table:

	***RecombiLISA* HBeAg ELISA**		
Ref. HBeAg EIA	**Positive**	**Negative**	**Total**
Positive	50	0	50
Negative	0	250	250
Total	50	250	300

Relative Sensitivity: 100% (95% CI: 94.9% - 100%)
Relative Specificity: 100% (95% CI: 98.9% - 100%)
Overall Agreement: 100% (95% CI: 99.1% - 100%)

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 in any other type of test kit as a substitute for the components in this kit.
5. Do not use serum derived from hemolyzed blood specimen 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 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 element to come into contact with the conjugate or TMB Substrate.
13. The TMB Substrate must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The TMB Substrate B must be stored in the dark.
14. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and TMB Substrate.
15. **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.**
16. **The microplate reader must be calibrated as per the manufacturer's instructions to ensure accurate determination of absorbance. A non-calibrated reader may lead to invalid test results.**
17. 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 HBeAg in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *RecombiLISA* HBeAg ELISA is limited to the qualitative detection of HBeAg in human serum or plasma. The intensity of color does not have a linear correlation with the antigen titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable HBeAg. 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 HBeAg present in the specimen is below the detection limits of the assay or the HBeAg 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 may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

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3. Dane DS, Cameron CH, Briggs M. Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. Lancet. 1970;1(7649):695-8.
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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
Σ N	Tests per kit	CONTROL -	Negative control
2	Do not reuse	TMB SUBS	TMB substrate
	Manufacturer	WASH BUFF	Wash buffer
	Date of manufacture	STOP SOLN	Stop solution
8°C 2°C	Store between 2-8°C		

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