# RecombiLISA

## HBeAb ELISA

IVD | REF E0721

- 96-well ELISA kit for the qualitative detection of HBeAb 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* HBeAb ELISA is a solid-phase enzyme-linked immunosorbent assay for the qualitative detection of hepatitis Be antibody (HBeAb) in human serum or plasma. It is intended for professional use only as an aid in the diagnosis of infection with HBV.

### INTRODUCTION

Hepatitis virus B (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]. HBeAb appears shortly after the disappearance of the antigen and is detectable for up to 2 years or more after resolution of the hepatitis.

### TEST PRINCIPLE

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

The *RecombiLISA* HBeAb ELISA is composed of two key components:

1) Solid microwells pre-coated with recombinant HBe antigen;
2) Liquid conjugates composed of anti-HBe antibody conjugated with horseradish peroxidase (HRP-HBeAb conjugate).

During the assay, the test specimen and HRP-HBeAb Conjugate are incubated simultaneously in the HBeAg coated microwells. The HBeAb, if present in the specimen, will compete with the constant amount of HRP-HBeAb for the limited amount of HBeAg coated on the microwell surface. Thus, the amount of HRP-HBeAb bound to the well (the HRP-complex) is inversely proportional to the concentration of HBeAb in the specimen.

Unbound material is then removed by washing. Upon addition of the TMB substrate to the microwells, the presence of the HRP-complex is shown by the development of a blue color resulting from a reaction between the enzyme and substrate. The reaction is quenched with the 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. Pipette capable of delivering 10 µL, 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 450nm wavelength is acceptable
3. Absorbent paper for blotting the microplate wells
4. Timer
5. Distilled or de-ionized water

**Materials and reagents provided with the kit**

| Item | Description | Quantity | Catalog |
|---|---|---|---|
| 1 | HBeAg Coated Microwells | 8 wells x 12 strips | E0721W |
| 2 | HBeAb Negative Control | 1 mL | E0721N |
| 3 | HBeAb Positive Control | 1 mL | E0721P |
| 4 | HRP–HBeAb Conjugate | 6 mL | E0721H |
| 5 | Wash Buffer (30x 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 | Package Insert | 1 | PI-E0721 |
| 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 reagents are brought to room temperature before opening. Reseal the microwells after removing the desired number of wells. Place unused wells in resealable plastic 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 whole blood specimen obtained by acceptable venipuncture technique.
- This kit is designed for use with serum or plasma specimen without additives only.
- If not tested immediately, the specimen can be stored at 2-8°C for up to 7 days. The specimens should be frozen at -20°C for longer storage. Avoid multiple freeze-thawing cycles. If a specimen is to be shipped, pack in compliance with federal regulations 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°C-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 strips needed and mark them on the ELISA Working Sheet with the appropriate information. Positive and Negative Controls require to 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 HBeAb Positive, Negative Control into the designated control wells, respectively.

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

*To ensure better precision, use pipette to handle solution.*

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 plate with a microplate sealer.
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 diluted 350 µL 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 diluted wash buffer. 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. Cover the plate with a sealer.
8. Incubate at 37°C in the 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 changes to a yellow color completely.**
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 HBeAb Positive, Negative Control and specimens, respectively | 50 µL |
| 3. | Add HRP-HBeAb Conjugate, except blank well | 50 µL |
| 4. | Gently shake and cover | 20 seconds |
| 5. | Incubate | 37°C, 30 minutes |
| 6. | Wash: manual or automatic | 5 times |
| 7. | Add TMB Substrate A and B | 50 µL + 50 µL |
| 8. | Cover and incubate in dark | 37°C, 10 minutes |
| 9. | Add Stop Solution<br>Gently mix | 50 µL<br>30 seconds |
| 10. | Read result | 450/620-690 nm<br>within 15 minutes |

### INTERPRETATION OF RESULTS

**A. Set up the cut-off value**

The cut-off value = N x 0.4+ P x 0.6

N: Mean OD of the negative control. If the OD of negative control is more than 1.5, use 1.5 for calculation. If less than 1.5, use actual value

P: Mean OD of the positive control

**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 HBeAb positive controls should be ≤ 0.10.
The mean OD value of the HBeAb negative controls should be ≥ 0.80.

Check the procedure and repeat assay if above conditions are 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 HBeAb in the specimen.
2. Specimens with cut-off < 1.00 are initially considered to be positive by the *RecombiLISA* HBeAb ELISA.
   Results just above the cut-off value (higher than 10% of the cut-off value) should be interpreted with caution. It is advisable to retest the corresponding specimens in duplicate when 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* HBeAb ELISA, subject to the limitation of the procedure, described below.
   If after retesting of the specimen, the absorbance value of the 2 duplicates are less than the cut-off value, the initial result is non-repeatable and the specimen should be considered negative with *RecombiLISA* HBeAb 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 TMB Substrate by oxidizing agents (bleach, metal ions, etc.)
   - Contamination of the Stop solution

## PERFORMANCE CHARACTERISTICS

**Clinical Performance**

A total of 300 specimens were collected from susceptible subjects and tested by a Chinese State Drug Administration (SDA) licensed reference EIA. Comparison for all subjects is shown in the following table:

| | ***RecombiLISA* HBeAb ELISA** | | |
|---|---|---|---|
| **Ref. HBeAb EIA** | **Positive** | **Negative** | **Total** |
| **Positive** | 43 | 0 | 43 |
| **Negative** | 0 | 267 | 267 |
| **Total** | 43 | 267 | 300 |

Relative Sensitivity: 100%, Relative Specificity: 100%, Overall Agreement: 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 devices.
3. Bring all reagents to room temperature (18°C-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 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. In the beginning of each incubation and after adding Stopping Solution, gently shake the microwells to ensure thorough mixing. Avoid the formation of air bubbles as which results in inaccurate absorbance values. Avoid splashing liquid while shaking the wells.
11. Don't allow the microplate to dry between the end of the washing operation and the reagent distribution.
12. The enzyme 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 B 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. **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.**
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 *RecombiLISA* HBeAb ELISA in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *RecombiLISA* HBeAb ELISA is limited to the qualitative detection of HBeAb in human serum or plasma. The intensity of color does not have linear correlation with the antibody titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable HBeAb. 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 HBeAb present in the specimen is below the detection limit of the assay or the HBeAb 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


1. Emanuel Rubin and John Farber. The liver and biliary system. Acute viral hepatitis P 721-729. Rubin E, Farber JL ed. Pathology 2nd ed. 1994. J.B. Lippincott, Philadelphia
2. 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.
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.
4. Magnius LO, Espmark A. A new antigen complex co-occurring with Australia antigen. Acta Pathol Microbiol Scand [B] Microbiol Immunol. 1972;80(2):335-7.


**Index of Symbols**

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


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PI-E0721 Rev. CP1.0
Effective date: 2019-09-20
English version