RecombiLISA

HBcAb ELISA

IVD | REF E0810

- 96-well ELISA Test for the qualitative detection of hepatitis B core antibody (HBcAb) 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* HBcAb ELISA is a competitive, solid-phase enzyme-linked immunosorbent assay for the qualitative detection of hepatitis B core antibody (HBcAb) 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 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].

Antibody to HBcAg (anti-HBc or HBcAb) appears shortly after HBsAg and before the appearance of detectable antibody to HBsAg, roughly at the time that serum ALT begins to rise. Anti-HBc also remains elevated for life and is a useful marker of the ongoing or previous HBV infection as HBcAg itself does not circulate freely in the serum of such infected persons[1,4].

TEST PRINCIPLE

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

The *RecombiLISA* HBcAb ELISA is composed of two key components:
1) Solid microwells pre-coated with recombinant HBc antigen (HBcAg);
2) Liquid conjugate composed of anti-HBc antibody conjugated with horseradish peroxidase (HRP-HBcAb Conjugate).

During the assay, the test specimen and HRP-HBcAb Conjugate are incubated simultaneously in the coated microwells. The HBcAb, if present in the specimen will compete with the controlled amount of HBcAb in the HRP conjugate for binding to the HBc antigen coated on the microwell surface. Because the HBcAb in the specimen and HRP conjugate compete, the amount of HRP-HBcAb and HBcAb from the specimen are inversely proportional.

Unbound materials are then removed by washing. The presence of the HRP- HBcAb Conjugate is shown by a blue color upon an additional incubation with TMB substrate. The reaction is quenched with the Stop Solution, and absorbance values are read using a spectrophotometer at 450 /620-690 nm.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	HBcAg Coated Microwells	12 wells x 8 strips	E0810W
2	HBcAb Negative Control	1 mL	E0810N
3	HBcAb Positive Control	1 mL	E0810P
4	HRP-HBcAb Conjugate	6 mL	E0810H
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	Product Insert	1	PI-E0810
Others	2 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 450nm wavelength is acceptable
3. Absorbent paper for blotting the microwells
4. Timer
5. Distilled or de-ionized water

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. All 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, 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	2.0 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

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 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.

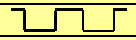
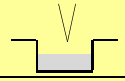
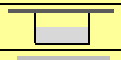
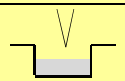
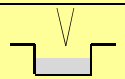
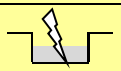
2.2 Control Wells: Add 50 µL of HBcAb Positive, Negative Control into the designated control wells, respectively.

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

3. Add 50 µL of HRP-HBcAb 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 into 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 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. 3.	Add HBcAb Positive, Negative Control, and specimens, respectively Add HRP-HBcAb Conjugate 	50 µL 50 µL
4. 5.	Gently shake Incubate 	20 seconds 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 0.4+ P x 0.6
N: Mean OD of the negative control
P: Mean OD of the positive 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.

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 HBcAb positive controls should be ≤ 0.1
The mean OD value of the HBcAb 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 HBcAb in the specimen.
2. Specimens with OD ratio ≤ 1.00 are initially considered to be positive by the *RecombiLISA* HBcAb ELISA. They should be retested in duplicate before final interpretation.
3. Results just high the cut-off value (higher than 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* HBcAb ELISA, subject to the limitation of the procedure, described below.
If after re-testing 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* HBcAb 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 stopping solution

PERFORMANCE CHARACTERISTICS

1. Clinical Performance

A total of 400 specimens were collected from susceptible subjects and tested by the *RecombiLISA* HBcAb ELISA and by a reference HBcAb ELISA. Comparison for all subjects is showed in the following table:

	***RecombiLISA* HBcAb ELISA**		
Ref. HBc Ab EIA	**Positive**	**Negative**	**Total**
Positive	58	0	58
Negative	0	342	342
Total	58	342	400

Relative Sensitivity: 100% (95% CI: 95.5% - 100%)
Relative Specificity: 100% (95% CI: 99.2% - 100%)
Overall Agreement: 100% (95% CI: 99.3% - 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 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. **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 HBcAb in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The *RecombiLISA* HBcAb ELISA is limited to the qualitative detection of HBcAb 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 *RecombiLISA* HBcAb ELISA. 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 HBcAb present in the specimen is below the detection limits of the assay, or the antibodies 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		Use by	
For *in vitro* diagnostic use only	IVD	Conjugate	CONJ
Catalog #	REF	Coated microwells	MICROWELLS
Lot number	LOT	Positive control	CONTROL +
Tests per kit	N	Negative control	CONTROL -
Do not reuse		Wash buffer	WASH BUFF
Manufacturer		TMB substrate	TMB SUBS
Date of manufacture		Stop solution	STOP SOLN
Store between 2-8°C	2°C 8°C		

CTK Biotech, Inc.
13855 Stowe Drive,
Poway, CA 92064, USA
Tel: 858-457-8698
Fax: 858-535-1739
E-mail: info@ctkbiotech.com

PI-E0810 Rev. CP
Date released: 2018-11-27
English version