Human HBcAb (hepatitis B virus core antibody) ELISA Kit

Catalogue No.: EH4107

Size: 96T

Reactivity: Human

Application: This immunoassay kit allows for the qualitative determination of HBcAbin human serum or plasma.

Storage: 2-8°C

Expiry Date: see kit label

Principle: Competitive

NOTE: FOR RESEARCH USE ONLY.

Kit Components

Item	Specifications(96T)	Storage
Micro ELISA Plate	8 ×12	2-8°C
HBcAb Negative Control	1ml ×1	2-8°C
HBcAb Positive Control	1ml ×1	2-8°C
HRP conjugated HBcAb	7ml ×1	2-8°C
Wash Buffer (20 x concentrate)	25 ml ×1	2-8°C
TMB substrate A	7ml ×1	2-8°C(Avoid Direct Light)
TMB substrate B	7ml ×1	2-8°C(Avoid Direct Light)
Stop solution	7ml ×1	2-8°C
Plate Sealer	3pieces	2-8°C
Instruction manual	1 copy	

Background

Hepatitis B virus（HBV） is composed of outer HBV surface antigen（HBsAg） and core antigen(HBcAg). HBcAb is a specific antibody produced by HBcAg stimulation. The generation of

Wuhan Fine Biotech Co., Ltd.

B9 Bld, High-Tech Medical Devices Park, No. 818 Gaoxin Ave. East Lake High-Tech Development Zone, Wuhan, Hubei, China(430206)

Tel: (0086)027-87384275 Fax: (0086)027-87800889 www.fn-test.com

HBcAb was earlier than HBsAb. Initially, HBcAb was dominated by IgM type. After about five weeks of illness, the antibodies change to IgG type. HBcAb is not a protective antibody, and it can exist for a long time with chronic HBV infection. In some individuals recovering from HBV infection, HBcAb remains in the blood for several years.

Principle of the Assay

This kit was based on Competitive ELISA. Recombined HBcAg was pre-coated onto 96-well plates. The test samples and HRP conjugated HBcAb were added to the wells, if there were any HBcAb in the samples, it would compete with a fixed amount of HRP conjugated HBcAb. TMB substrates were used to visualize HRP enzymatic reaction. It was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The optical density of developed color is read with a suitable photometer at 450nm with a selected reference wavelength within 650 nm.

Precautions for Use

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manuf acturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

Wuhan Fine Biotech Co., Ltd.

B9 Bld, High-Tech Medical Devices Park, No. 818 Gaoxin Ave. East Lake High-Tech Development Zone, Wuhan, Hubei, China(430206)

Tel: (0086)027-87384275 Fax: (0086)027-87800889 www.fn-test.com

Material Required But Not Supplied

1. Microplate reader (wavelength: 450nm)
2. 37℃ incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Manual Washing

Discard the solution in the plate without touching the sidewalls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a total of THREE washes.

Automated Washing

Aspirate all wells, wash plate THREE times with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 minute.

Sample Collection and Storage

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C for long term. Avoid multiple freeze-thaw cycles.

- Serum: Coagulate the serum at room temperature (about 1 hour). Centrifuge at approximately 1000 × g for 15 min. Analyze the serum immediately or aliquotand store at -20°C.
- Plasma: Collect plasma with heparin,sodium citrateor EDTA as the anticoagulant. Centrifuge for 15min at 2-8°C at 1500 xg within 30 min of collection. For eliminating the platelet effect, suggesting that further centrifugation for 10 min at 2-8°C at 10000 xg. Analyze immediately or aliquotand store frozen at -20°C.

Wuhan Fine Biotech Co., Ltd.

B9 Bld, High-Tech Medical Devices Park, No. 818 Gaoxin Ave. East Lake High-Tech Development Zone, Wuhan, Hubei, China(430206)

Tel: (0086)027-87384275 Fax: (0086)027-87800889 www.fn-test.com

Note: Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Hemolyzed samples are not suitable for use in this assay.

Wash Buffer Preparation:

Dilute 25mL of Concentrated Wash Buffer to 500 mL of Wash Buffer with deionized or distilled water.

Assay Procedure

1. Bring all reagents to room temperature before use.
2. Label the sample wells, 2 Negative Controls, 2 Positive Controls and 1 blank well(Sample and enzyme-labeled antibodies were not added to the blank Well, and the remaining steps were the same).
3. As a basis for clinical diagnosis, the protox serum sample must be diluted at 1:30 and then tested, and the dilution buffer is saline or 10mM PBS. As a basis for epidemiological investigation, the original serum test was used.
4. Add 50 µL Sample, Negative Controls and Positive Controls to Corresponding wells.
5. Add 50 µL HRP- HBcAb to each well.
6. Seal the plate with a cover and incubate at 37°C for 60 min. Balance at room temperature for 5 min.
7. Remove the cover, and wash plate 5 times with Wash buffer and each time let the wash buffer stay in the wells for 0.5-1 min.
8. Add 50µlof TMB substrate A and 50µlofTMB substrate B into each well. Gently tap the plate to ensure thorough mixing. Cover the plate and incubate at 37°C in dark for 15 min. And the shades of blue can be seen in the Positive Controls. Negative Controlswells show no obvious color.
9. Add 50µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

Wuhan Fine Biotech Co., Ltd.

B9 Bld, High-Tech Medical Devices Park, No. 818 Gaoxin Ave. East Lake High-Tech Development Zone, Wuhan, Hubei, China(430206)

Tel: (0086)027-87384275 Fax: (0086)027-87800889 www.fn-test.com

10. The OD values of each well were determined with a microplate reader with a single wavelength of 450 nmora double wavelength of 450 nm/630 nm, The measurement is completed within 30 minutes.

Data Analysis

Calculation of Results

Sample is undiluted ， Cutoff Value(C.O.) = NCx×0.2

Sample is diluted， Cutoff Value(C.O.) = NCx×0.5

NCx: Mean Absorbance of Negative Control

Note:

1. Sample with absorbance values S /C.O. > 1 are NON-REACTIVE and are considered NEGATIVE for HBcAb.

   Sample with absorbance value S /C.O. ≤ 1 are considered POSITIVE for HBcAb.

2. All samples, waste liquids and wastes should be treated according to the source of infection.

Wuhan Fine Biotech Co., Ltd.

B9 Bld, High-Tech Medical Devices Park, No. 818 Gaoxin Ave. East Lake High-Tech Development Zone, Wuhan, Hubei, China(430206)

Tel: (0086)027-87384275 Fax: (0086)027-87800889 www.fn-test.com