Hepatitis B virus core antigen Ab ELISA Kit

Catalog Number KA0288

96 assays

Version: 14

Intended for research use only

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Introduction

Intended Use

The Hepatitis B virus core antigen Ab ELISA kit is an enzyme immunoassay kit for qualitative detection of Antibody to Hepatitis B virus core antigen (IgG Anti-HBc) in human serum or plasma.

Principle of the Assay

The Hepatitis B virus core antigen Ab ELISA kit is a solid-phase enzyme immunoassay (ELISA= enzyme-linked immune assay) - based on a competitive principle. The solid phase of the microtiter plate is made of polystyrene wells coated with HBcAg and the liquid phase of human peroxidase conjugated Anti-HBc. When a serum or plasma specimen containing Anti-HBc is added to the HBcAg-coated wells together with the human peroxidase conjugated Anti-HBc and incubated, a competition will take place for the binding to the HBcAg on the wells. (HBcAg)-(Anti-HBc • Peroxidase) complex and/or (HBcAg)-(Anti-HBc) complex will form on the wells. After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. Due to the competitive principle a color develops inversely proportional to the amount of Anti-HBc bound to HBcAg deriving from the specimen. The Peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450 nm with a selected reference wavelength within 620 to 690 nm.

The above test principle is shown also in the following.

- Specimen containing Anti-HBc:

1. Plate well (HBcAg) + Specimen (Anti-HBc) + Anti-HBc • HRPO ⟶ HBcAg • Anti-HBc and /or HBcAg • Anti-HBc • HRPO.
2. HBcAg • Anti-HBc and /or HBcAg • Anti-HBc • HRPO + TMB solution (colorless) ⟶ colorless to light blue.
3. Colorless to light blue + 2 N H_2SO_4 ⟶ colorless to light yellow.

- Specimen without Anti-HBc:

1. Plate well (HBcAg) + Specimen + Anti-HBc • HRPO ⟶ HBcAg • (Anti-HBc • HRPO).
2. HBcAg • (Anti-HBc • HRPO) + TMB solution (colorless) ⟶ light blue to blue.
3. Light blue to blue + 2 N H_2SO_4 ⟶ light yellow to yellow.

General Information

Materials Supplied

List of component

Component	Description	Amount
HBcAg Plate	Microtiter plate coated with HBcAg.	1 plate
Anti-HBc • Peroxidase Solution	Anti-HBc (human) • Peroxidase conjugate dissolved in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	8 mL
Anti-HBc Positive Control	Anti-HBc and HBsAg positive serum in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1.5 mL
HB Negative Control	Serum non-reactive for HBV markers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	2 mL
TMB Substrate Solution A	3,3',5,5'-tetramethylbenzidine (TMB) solution.	12 mL
TMB Substrate Solution B	Citrate acid buffer containing H_2O_2.	12 mL
Conc. Washing Solution D (20x)	Concentrated phosphate buffer with Tween-20	58 mL
2 N H_2SO_4	2 N H_2SO_4 (Sulfuric Acid)	12 mL

Accessories: (provided as needed)

Component	Amount
Adhesive slips	X1
Black cover	X1

Storage Instruction

- ✓ The kit must be stored at 2-8℃. Do not freeze.
- ✓ Strips of the plate should be used within one month after open the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and tapped the opening tightly.
- ✓ Return the reagents to 2-8℃ immediately after use.
- ✓ Washing Solution D (20X) Concentrate should be stored at room temperature to avoid crystallization. Conc. Washing Solution D (20X) is stored at +2 to +8°C, which can cause crystallization. If the crystal has been precipitated before use, warm up the solution in a 37℃ water bath till crystal dissolved.

Materials Required but Not Supplied

- ✓ 50 µL, 100 µL micropipettes and tips are needed
- ✓ Incubator or water bath with temperature control at 37 ± 1°C
- ✓ Plate washing equipment.
- ✓ ELISA microwell reader
- ✓ Fully automatic EIA micro-plate analyzer is optional. User should validate the automatic EIA micro-plate analyzer in combination with the kit.

Precautions for Use

Procedural Guidelines

- ✓ This kit is for research use only.
- ✓ Bring all kit reagents and samples to room temperature (20-30℃) and mix gently before use.
- ✓ Do not use kit beyond its expiration date.
- ✓ Do not interchange reagents between different lots.
- ✓ Reagents must be protected from microbial contamination.
- ✓ The positive and negative control sera have been inactivated, however, for safety reason, they must be treated as infectious material.
- ✓ Do not smoke or eat in areas where specimens or reagents are handled.
- ✓ Do not pipette by mouth.
- ✓ Wear gloves when handling reagents or specimens, and wash hands thoroughly afterwards.
- ✓ Infectious specimens and nonacid containing spills should be wiped up thoroughly with 5% sodium hypochlorite.
- ✓ All waste materials should be properly disinfected before disposal. Both liquid and solid waste should be autoclaved for at least 1 hour at 121℃. Solid waste can also be incinerated. Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%. Liquid waste containing acid must be neutralized before similar treatment and should stand for 30 minutes to obtain effective disinfection.
- ✓ TMB substrate solution A contains dimethyl sulfoxide, an irritant to skin and mucous membranes. Avoid contact of TMB substrate solution and sulfuric acid with skin and mucous membranes.

Assay Protocol

Reagent Preparation

- Preparation of Washing solution
  Dilute Washing Solution D (20x) Concentrate with distilled or de-ionized water to 1:20 dilution. Do not use tap water.
- Plate Washing Procedure

✓ AUTOMATIC OR SEMI-AUTOMATIC PLATE WASHER
Any commercial automatic microplate washer or other liquid aspirating/dispensing devices can be used for washing purpose. The user should test the devices to determine the proper volume of water and wash cycles to insure proper washing. We suggested 6 wash cycles with at least 350 µL per well per wash and soaking for 10 seconds is necessary.

✓ MANUAL PLATE WASH
Cover the reaction plate with an absorbent paper. Invert the plate and allow the liquid absorb onto the absorbent paper, then return the plate back to upright position. Fill each well with 350 µL of washing buffer. Aspirate the water after soaking 10 seconds. Repeat this procedure 6 times. Blot dry by inverting the plate and tapping firmly onto absorbent paper. All residual washing buffer should be blotted dry.
WARNING: Improper washing can cause false results.

Sample Preparation

- Specimen Collection and Preparation for Analysis

✓ Either human serum or plasma can be used with this assay kit. Whole blood specimens should be separated as soon as possible in order to avoid hemolysis. Any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen should be removed prior to use.

✓ Specimens must be stored at 2-8℃ and avoided heat-inactivation to minimize deterioration. For long-term storage, specimens should be frozen below -20℃. Avoid multiple freeze-thaw procedures. Storage in self-defrosting freezer is not recommended.

✓ Pretreatment of sample is needed if the sample containing substances that may interfere with the assay.

✓ Frozen specimens must be thoroughly thawed and mixed homogenously before test.

✓ The specimen must not contain any AZIDE compounds which can inhibit the peroxidase activity of the conjugate.

Assay Procedure

1. Bring all reagents and specimens to room temperature (20-30℃) before assay. Adjust water bath or incubator to 37±1℃.
2. Reserve 2 wells for blanks. Add 50 µL of each control or specimen to appropriate wells of reaction plate

(3 Negative Controls and 2 Positive Controls).

NOTE: Use a new pipette tip for each sampling to avoid cross contamination.

3. Add 50 µL of Anti-HBc • Peroxidase Solution to each well except 2 blanks.

   NOTE: Do not touch the well wall to avoid contamination.

4. Gently tap the plate.
5. Remove the protective backing from the Adhesive Slip and press it on the reaction plate, so that it is tightly sealed.
6. Incubate the reaction plate in a 37±1℃ water bath or incubator for 1 hour.
7. At the end of the incubation period, remove and discard the Adhesive Slip and wash plate by following "PLATE WASHING PROCEDURES".
8. Choice one of the following two methods for color development:

   NOTE: TMB Substrate Solution A should be colorless to light blue, otherwise, should be discarded. The mixture of TMB Substrate Solution A and B should be used within 30 minutes after mix. The mixture should be avoided from intense light.

   ✓ Mix equal volume of TMB Substrate Solution A and B in a clean container immediately prior to use. Add 100 µL of the mixture solution to each well including 2 blank wells.

   ✓ Add 50 µL of TMB Substrate Solution A first, then add 50 µL of TMB Substrate Solution B into each well including 2 blanks. Mix well gently.

9. Cover the plate with Black Cover and incubate at room temperature (20-30℃) for 30 minutes.
10. Stop the reaction by adding 100 µL 2 N H_2SO_4 to each well including 2 blanks.
11. Determine absorbance of Controls and test specimens within 15 minutes with a precision spectrophotometer at 450 nm or 450/650 nm (450 nm reading wavelength with 650 nm reference wavelength). Use the lighter color of two blank wells to blank the spectrophotometer.

NOTE: The blanks should be colorless to light yellowish in color; otherwise, the test results are invalid. In this case the tests must be repeated. The absorbance value of blank must be less than 0.100.

Data Analysis

Calculation of Results

Calculation of the NCx (Mean Absorbance of Negative Control)

Example:

Sample No.	Absorbance
1	0.939
2	0.944
3	0.925

NCx = (0.939 + 0.944 + 0.925) / 3 = 0.936

NCx must be≥ 0.4, otherwise, the test run is invalid.

Calculation of the PCx (Mean Absorbance of Positive Control)

Example:

Sample No.	Absorbance
1	0.068
2	0.052

PCx = (0.068 + 0.052) /2 = 0.060

PC x must be ≤ 0.1, otherwise, the test run is invalid.

Calculation of the N - P Value

N - P = NC x – PC x

Example:

N – P = 0.936 – 0.060 = 0.876

N - P Value must be ≥ 0.3, otherwise, the test run is invalid.

Calculation of the Cutoff Value

Cutoff Value = 0.4 NCx + 0.6 PCx

Example:

Cutoff Value = (0.4 x 0.936) + (0.6 x 0.060) = 0.410

Calculation of the Retest Range

Retest Range = Cutoff Value ± 10%

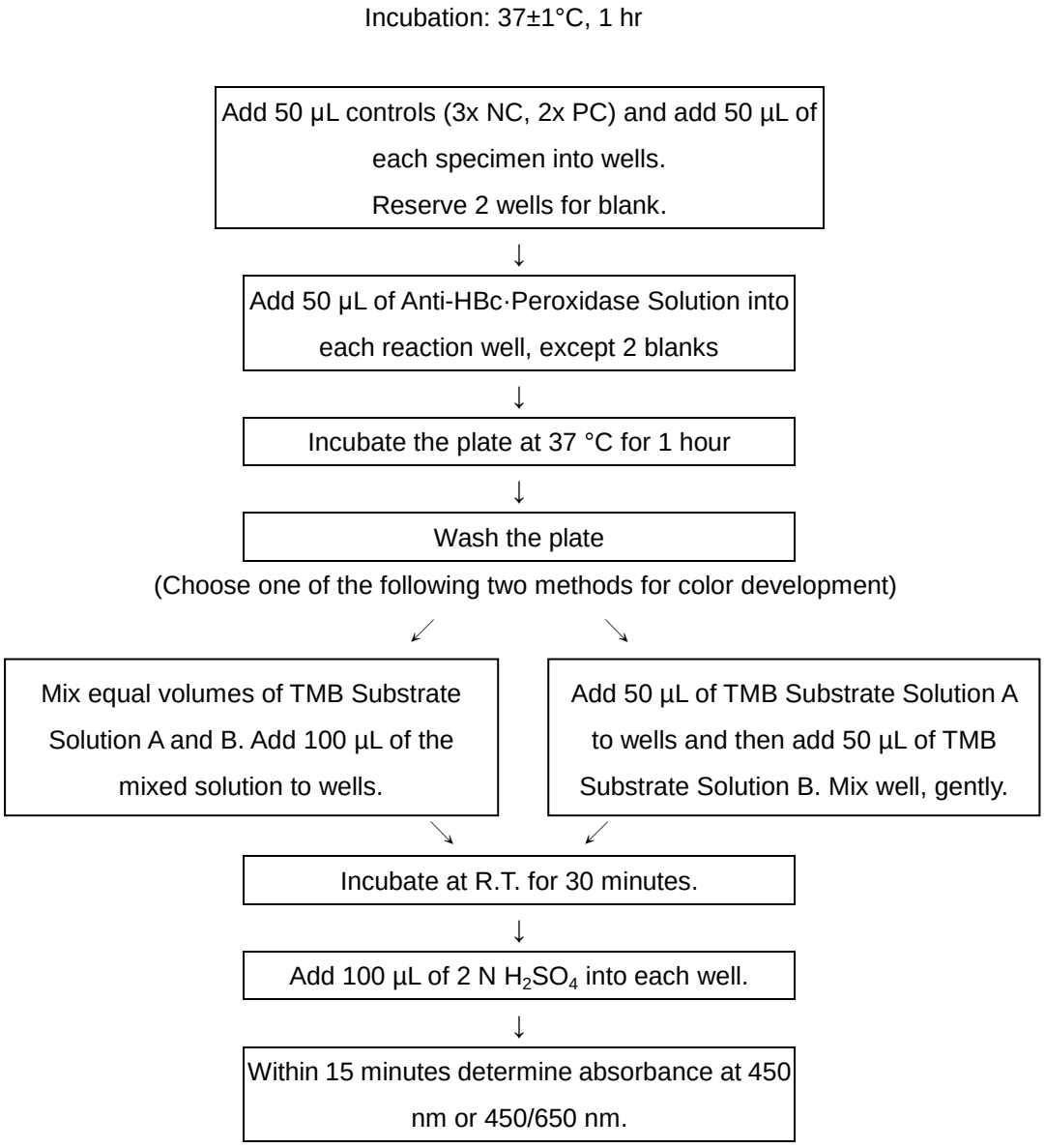
Example: Cutoff Value = 0.410

Retest Range = (0.410 - 0.041) to (0.410 + 0.041) = 0.369 to 0.451

Note:

- ✓ *Specimens with absorbance values greater than the cutoff value are considered negative for Anti-HBc.*
- ✓ *Specimens with absorbance value less than or equal to the cutoff value are considered positive for Anti-HBc.*
- ✓ *If the signal/cut-off ratio is within retest range, the test must be repeated in duplicate and interpreted as above.*

- Flow chart of the test procedure



Resources

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Plate Layout

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