Hepatitis B virus IgM ELISA Kit

Catalog Number KA0289

96 assays

Version: 03

Intended for research use only

www.abnova.com

Table of Contents

Introduction

Background

The hepatitis B virus (HBV) consists of an external envelope (HBsAg) and an inner core (HBcAg). In acute HBV infection, IgM antibodies to HBcAg (Anti-HBc IgM) are detectable in serum or plasma shortly after the onset of viral replication and remain in the circulation for about 7 to 17 weeks. The detection of anti-HBc IgM antibodies is used, in conjunction with HBsAg determination, as indicative marker of the phase of the infection and for the monitoring of patients under treatment with interferon. High anti-HBc IgM titers can be found in acute HBV infection and in attacks during chronic hepatitis B. The level of anti-HBc IgM decreases throughout the course of infection. However, low levels of anti-HBc IgM may persist for over a year after infection in some patients and are found occasionally in chronic carriers.*1-6

Principle of the Assay

The Hepatitis B virus IgM ELISA kit is based on Second Antibody Sandwich Princple. The reaction precesses are summaruzed as follos:

1. Plate (Anti-human IgM) + specimen (containing human Anti-HBc IgM) → Anti-human IgM • Human Anti-HBc IgM
2. Anti-human IgM • human Anti-HBc IgM + HBcAg + Anti-HBc • HRPO → Anti-human IgM • human Anti- HBc IgM • HBcAg • (Anti-HBc • HRPE) sandwich complex
3. Anti-human IgM • human Anti-HBc IgM • HBcAg • (Anti-HBc • HRPO) sandwich complex + TMB Solution (colorless) → light blue to blue color
4. Light blue to blue color + 2N H_2SO_4 → light yellow to yellow color absorbing at 450nm

General Information

Materials Supplied

List of component

Component	Description	Amount
Anti-IgM Microtiter Plate	One microtiter plate (removable strips) with 96 wells coated with Anti-human IgM.	1 plate
Anti-HBc • Peroxidase Solution	Anti-HBc (human) • Peroxidase (horseradish) conjugate in buffer with protein stabilizer. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1bottle, 5 ml
Anti-HBc IgM Positive Control	Human Anti-HBc IgM in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1 bottle, 1.5 ml
Anti-HBc IgM Negative Control	Normal human serum containing protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1 bottle, 2 ml
Specimen Diluent	Buffer containing protein stabilizers. Preservatives: 0.003%Gentamycin and 0.01% Thimerosal.	2 bottle 35 ml
HBcAg Reagent	HBcAg in buffer containing protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1 bottle, 5 ml
TMB Substrate Solution A	0.6 mg/ml of 3,3',5,5'-tetramethylbenzidine (TMB) in an organic base.	1 bottle, 10 ml
TMB Substrate Solution B	Citric acid buffer containing 0.03% H_2O_2.	1 bottle, 10 ml
Conc. Washing Solution D (20x)	Phosphate buffer with Tween-20.	1 bottle 50 ml
2N Sulfuric acid	2N H_2SO_4 (Sulfuric acid)	1 bottle 10 ml
Black Cover		
Adhesive Slips		

Storage Instruction

- ✓ The kit must be stored at 2-8℃. Do not freeze.
- ✓ Washing Solution D (20X) Concentrate and 2N Sulfuric acid should be stored at room temperature to avoid 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

- ✓ 5µl, 50µl and 100 µl micropipettes and tips are needed
- ✓ Incubator or water bath with temperature control at 37 ±1℃
- ✓ Plate washing equipment.
- ✓ ELISA microwell reader: precision ELISA reader capable for 450nm wavelength reading is necessary
- ✓ Fully automatic EIA micro-plate analyzer (optional)

Precautions for Use

- Procedural Guidelines
- ✓ Bring all kit reagents and samples to room temperature (+20 to +30℃) and mix carefully before use.
- ✓ Do not use reagent beyond its expiration date.
- ✓ Do not interchange reagents between different lots.
- ✓ Reagents must be protected from microbial contamination.
- ✓ Positive control is made of low infectious HBsAg and IgM Anti-HBc positive serum, 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 mouse.
- ✓ Wear gloves when handling reagents or specimens, and wash hands thoroughly afterwards.
- ✓ Infectious specimens and non-acid 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 maintaining +121 ℃ for at least 1 hour. Solid waste can also be incinerated.
- ✓ Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%.
- ✓ Liquid wastes must be neutralized before treatment with sodium hypochlorite as mentioned above 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

Plate washing procedure

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.

- 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 0.35 ml per well per wash and soaking for 10 seconds is necessary.

- Automatic or semi-automatic plate washer

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 0.35 ml of washing buffer. Aspirate the wash 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 serum or plasma can be used with this kit. Whole blood specimen 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 to +8℃ and avoided heat-inactivation to minimize deterioration. For long-term storage, specimens should be frozen below -20℃. Storage in self-defrosting freezers is not recommended.
- ✓ Frozen specimens must be thoroughly thawed and mixed homogenously before test.
- ✓ Avoid multiple freeze-thaw procedures

Assay Procedure

1. Bring all reagents and specimens to room temperature (+20 to +30 ℃) before assay. Swirl the reagents gently before use. Adjust water bath or incubator to +37±1℃.
2. Make 1:100 dilution of each specimen:
   Label the tubes for specimen dilution, one tube for each specimen. Add 0.5 ml of specimen diluent and 5 µl of each specimen to appropriate tube and mix well.
   Note:
   A. Do not dilute control
   B. Use a new pipette tip for each sampling to avoid cross-comtamination.
3. Reserve 2 well for Blank. Add 100 µl of each control (3 Negative Controls and 2 Positive Controls) and 100 µl of Specimen Diluent to appropriate wells of reaction plate.
4. Add 5 µl of diluted specimen into each well containing Specimen Diluent, respectively.
   Note:
   A. Do not dilute control
   B. Use a new pipette tip for each sampling to avoid cross-comtamination.
5. Gently tap the plate.
6. Remove the protective backing of the adhesive slip and press it on the reaction plate, so that it is tightly sealed.
7. Incubate the plate at +37℃ for 1 hour.
8. At the end of the incubation period, remove and discard the Adhesive Slip and wash plate by “Plate washing procedure”.
9. Add 50 µl of HBcAg reagent and 50 µl of Anti-HBc-Peroxidase solution into each reaction well, except 2 blanks.
10. Repeat steps 5 and 6.
11. Incubate the plate at +37 + 1℃ ℃ for 1 hour.
12. At the end of the incubation period, remove and discard the adhesive slip, wash the plate by “Plate washing procedure”.
13. Select 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.
    A. Mix equal volumes 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 the blank well.
    B. Add 50 µl of TMB Substrate Solution A first, and then add 50 µl of TMB Substrate Solution B into each well including 2 blanks. Mix well gently.

14. Cover the plate with the black cover and incubate at room temperature for 30 minutes.
15. Stop the reaction by adding 100 µl of 2N H_2SO_4 to each well including 2 blanks.
16. Determine the absorbance of controls and test specimens within 15 minutes with a precision photometer at 450 nm or 450/650 nm (450 nm reading wavelength with 650 nm reference wave length). Use lighter color of two blank wells to black spectrophotometer.

    Note:

    The color of the blank should be colorless to light yellow; otherwise, the test result must be repeated.

Data Analysis

Calculation of Results

1. Calculation of the NCx (Mean Absorbance of Negative Control)

Example:

Sample No.	Absorbance
1	0.068
2	0.072
3	0.070

NCx = (0.068+0.072 + 0.070) / 3 = 0.070

NCx must be ≤ 0.1, otherwise the test is invalid.

2. Calculation of PCx (Mean Absorbance of Positive Control)

Example:

Sample No.	Absorbance
1	1.612
2	1.613

PCx = (1.612 + 1.613) /2 = 1.613

PCx must be ≥ 0.4, otherwise the test is invalid.

3. Calculation of P-N Value

P-N = PCx - NCx

Example:

P - N = 1.613 – 0.070 = 1.543

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

4. Calculation of the Cutoff Value

Cutoff Value = NCx + (0.25 PCx)

Example:

Cutoff Value = 0.070 + (0.25 x 1.613) = 0.473

5. Calculation of the Retest Range

Retest Range = Cutoff Value ±10%

Example: Cutoff Value = 0.473

Retest Range = (0.473 - 0.047) to (0.473 +0.047) = 0.426 to 0.520

- Result interpretation

1. Specimen with absorbance values
   GREATER than or EQUAL to the Cutoff Value are considered POSITIVE for IgM Anti-HBc.
2. Specimen with absorbance values
   Less than the Cutoff Value are considered NON-REACTIVE for IgM Anti-HBc.
3. If the data is within the RETEST RANGE, the must be repeated and interpreted as above.

- Flow chart of the test procedure

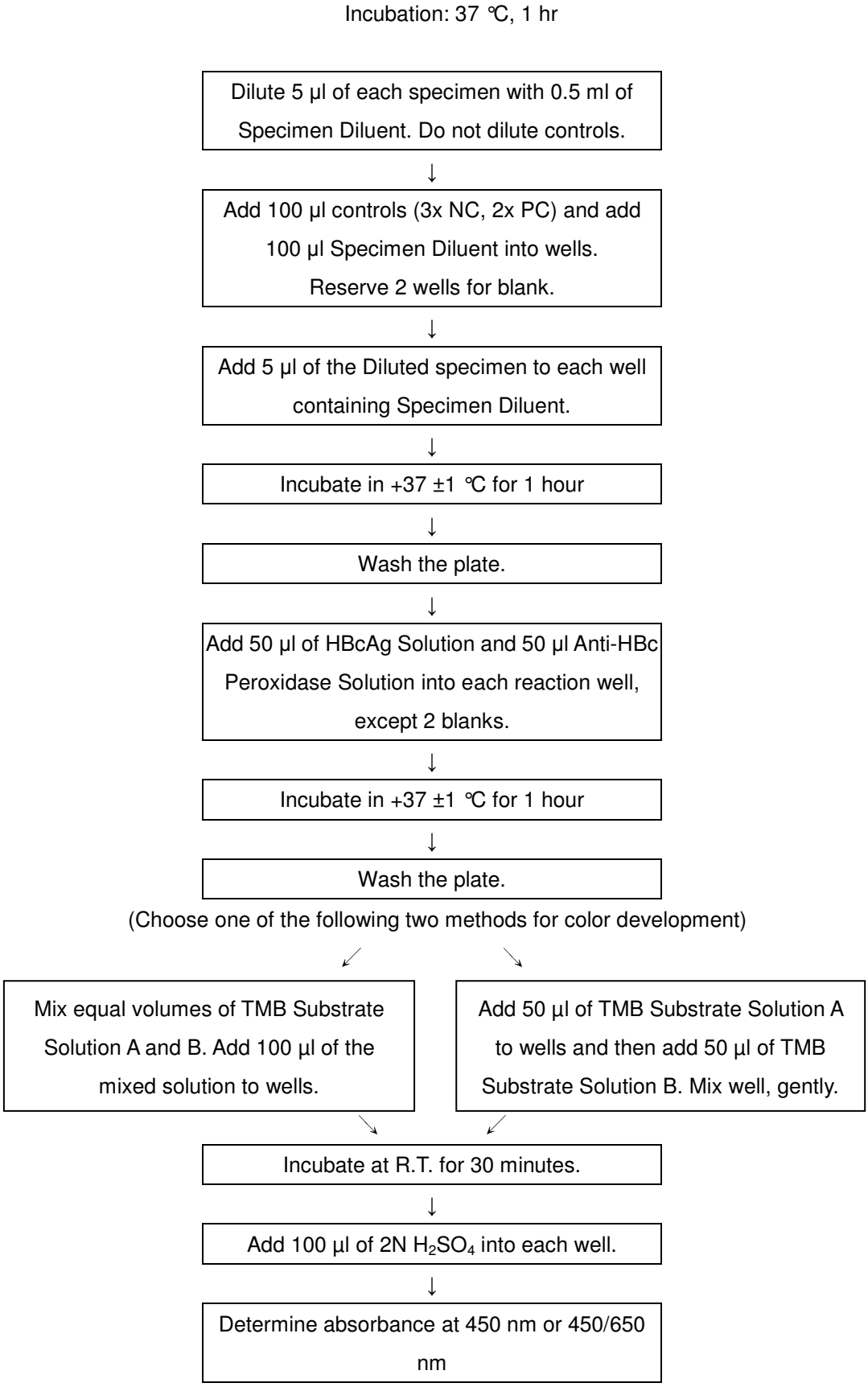

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H