HBeAg and HBeAg Ab ELISA Kit

Catalog Number KA0290

96 assays

Version: 04

Intended for research use only

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Introduction

Intended Use

The HBeAg and HBeAg antibody ELISA Kit is an enzyme immunoassay used for the detection of HBeAg and Anti-HBe antibody in human serum or plasma.

Principle of the Assay

1. For HBeAg detection

For HBeAg detection, HBEAG AND HBEAG ANTIBODY ELISA KIT (TMB) adopts the "sandwich principle" (Antibody • Antigen • Antibody) as the basis of the assay. When Anti-HBe coated wells and Anti-HBe • HRPO conjugate are incubated with specimens containing HBeAg, (antibody)-(antigen)-(antibody • HRPO) complexes are formed on the wells. After washing to remove unbound materials, TMB substrate is added and color develops in proportion to the amount of HBeAg bound. The color development is stopped by adding 2N sulfuric acid. The Optical Density of developed color is read with a suitable spectrophotometer against 450 nm/620-690 nm*.

The above description is shown in the following figure.

A. Specimen containing HBeAg:
   a. (Plate well)-(Anti-HBe) + Specimen (HBeAg) + Anti-HBe • HRPO
      → (plate well)-Anti-HBe • HBeAg • (Anti-HBe • HRPO) sandwich complex
   b. Sandwich complex + TMB substrate solution → Light blue to blue color
   c. Add 2N H_2SO_4 to stop the color development →Read OD at 450 nm/620-690 nm*

B. Specimen without HBeAg:
   a. (Plate well)-(Anti-HBe) + Specimen (no HBeAg) + Anti-HBe • HRPO → (plate well)-Anti-HBe
   b. (plate well)-Anti-HBe + TMB substrate solution → Colorless to light blue color
   c. Add 2N H_2SO_4 to stop the color development →Read OD at 450 nm/620-690 nm*

2. For Anti-HBe detection

For Anti-HBe detection, HBEAG AND HBEAG ANTIBODY ELISA KIT (TMB) adopts the "Neutralization Principle".

When specimen is incubated with Neutralizing Solution for Anti-HBe in the well(s) coated with Anti-HBe, The HBeAg from the Neutralizing Solution for Anti-HBe will be neutralized by Anti-HBe in the specimen. The more the concentration of Anti-HBe, the less the concentration of remaining HBeAg, and finally the lower the Optical Density developed. The above description is shown in the following figure.

A. Specimen containing Anti-HBe:
   a. (plate well)-Anti-HBe + Specimen (Anti-HBe) + Neutralizing Solution (HBeAg)
      → (Plate well)-Anti-HBe • HBeAg and HBeAg • Anti-HBe

After washing, only (Plate well)-Anti-HBe • HBeAg remains on the plate well(s).

b. (Plate well)-Anti-HBe • HBeAg + Anti-HBe • HRPO

→ (Plate well)-Anti-HBe • HBeAg • (Anti-HBe • HRPO) sandwich complex

c. Sandwich complex + TMB substrate solution

→ Light blue to colorless

d. Add 2N H_2SO_4 to stop the color development

→ Read OD at 450 nm/620-690 nm*.

B. Specimen without Anti-HBe:

a. Plate well (Anti-HBe) + Specimen (without Anti-HBe) + Neutralizing Solution (HBeAg)

→ (Plate well)-Anti-HBe • HBeAg

b. (Plate well)-Anti-HBe • HBeAg + Anti-HBe • HRPO

→ (Plate well) - Anti-HBe • HBeAg • (Anti-HBe • HRPO) sandwich complex

c. Sandwich complex + TMB substrate solution → blue color

d. Add 2N H_2SO_4 to stop the color development →Read OD at 450 nm/620-690 nm*.

General Information

Materials Supplied

List of component

Component	Description	Amount
Anti-HBe Plate	One microtiter plate coated with antibody to HBeAg（Anti-HBe）.	1 plate
Anti-HBe • Peroxidase Solution	Containing anti-HBe • Peroxidase (horseradish) conjugate dissolved in protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1 bottle, 11 mL
HBeAg Positive Control	Containing HBeAg positive serum diluted in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1 bottle,1.5 mL
Anti-HBe Positive Control	Containing Anti-HBe positive serum dissolved in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1 bottle,1.5 mL
HB Negative Control	Containing normal human serum, which is free of HBeAg, Anti-HBe and HBsAg. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1 bottle, 2 mL
Neutralizing Solution for Anti-HBe	Containing HBeAg positive serum diluted in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.	1 bottle, 7 mL
TMB Substrate Solution A	3,3',5,5'-tetramethylbenzidine (TMB) in an organic base.	1 bottle,12 mL
TMB Substrate Solution B	Citrate Acid Buffer containing 0.03% H_2O_2.	1 bottle,12 mL
Conc. Washing Solution D (20X)	Concentrated Phosphate buffer with Tween-20	1 bottle,58 mL
2N Sulfuric Acid	2 N H_2SO_4	1 bottle,12 mL

Accessories: (provided as needed)

Component	Amount
Adhesive slips	X1
Black cover	X1

Storage Instruction

- ✓ The kit must be stored at 2-8°C. 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°C immediately after use.
- ✓ Washing Solution D (20X) Concentrate should be stored at room temperature to avoid crystallization. If the crystal has been precipitated before use, warm up the solution in a 37°C 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°C ± 1°C.
- ✓ Plate washing equipment.
- ✓ ELISA micro-plate reader:
  Precision ELISA Reader capable for 450 nm wavelength reading is necessary.

Precautions for Use

- ✓ This kit is for professional use only.
- ✓ This reagent kit is for research use only.
- ✓ Bring all kit reagents and samples to room temperature (20 to 30°C) 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.
- ✓ HBeAg Positive Control and Neutralizing Solution for Anti-HBe are made of HBeAg positive serum, and are considered the etiological agent, therefore it 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°C. 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 cotact of TMB substrate solution and sulfuric acid with skin and mucous membrane.

Assay Protocol

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. Also clots must be removed.

- Stability and storage:

✓ Specimens must be stored at 2-8°C and avoided heat-inactivation to minimize deterioration. For long-term storage, they should be frozen below -20°C. Storage in self-defrosting freezer is not recommended.

✓ Avoid multiple freeze-thaw procedures.

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

✓ Do not use heat-inactivated specimen.

Assay Procedure

• Plate Washing Procedure

NOTE: Dilute Washing Solution D (20X) Concentrate with distilled or deionized water to 1:20 dilution. Do not use tap water.

a. 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.

b. 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.

- HBeAg Detection

1. Bring all reagents and specimens to room temperature (20 to 30°C) before assay. Adjust water bath or incubator to 37±1°C.
2. Reserve 2 wells for blanks. Add 100 µL of Control (3 Negative Controls and 2 HBeAg Positive Control) and 100 µL of each Specimen into wells, respectively.
   Note: Use an individual tip for each sample to avoid cross-contamination.
3. Gently tap the plate.
4. Remove the protective backing from the adhesive slip and press it onto the plate, so that it is tightly sealed.
5. Incubate the plate in a 37±1°C incubator or water bath for 1 hour.
6. At the end of the incubation period, remove and discard the adhesive slip and wash the plate by following the “Plate Washing Procedure”.
7. Add 100 µL of Anti-HBe • Peroxidase Solution into each reaction well except blanks.
   Note: Do not touch the edge of well to avoid false results.
8. Repeat steps 3 and 4.
9. Incubate the reaction plate in a 37±1°C incubator or water bath for 1 hour.
10. Repeat step 6 to wash the plate.
11. 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 afer 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 2 blank wells.
    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.
12. Cover the plate with a black cover and incubate at room temperature for 30 minutes.
13. Stop the reaction by adding 100 µL of 2 N H_2SO_4 to each well including 2 blanks.
14. 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 wavelength). Use the blank well to blank photometer. Use the lighter color of two blank wells to blank spectrophotometer.

Note: The color of the blank should be colorless to pale yellowish; otherwise, the test must be repeated.

Substrate blank: absorbance value must be less than 0.100.

- Anti-HBe Detection

1. Bring all reagents and specimens to room temperature (20 to 30°C) before assay. Adjust water bath or incubator to 37±1°C.
2. Add 50 µL of control (3 Negative Controls and 2 Anti-HBe Positive Controls) and 50 µL of each Specimen into wells, respectively. Reserve 2 wells for blanks.
3. Add 50 µL of Neutralizing Solution for Anti-HBe into each well except blanks.
   Note: Use an individual tip for each sample to avoid cross-contamination
4. Gently tap the plate.
5. Remove the protective backing from the adhesive slip and press it on the plate, so that it is tightly sealed.
6. Incubate the plate in a 37±1°C incubator or water bath for 1 hour.
7. At the end of the incubation period, remove and discard the adhesive slip and wash the plate by following the “Plate Washing Procedure”.
8. Add 100 µL of Anti-HBe • Peroxidase Solution into each reaction well except 2 blanks.
   Note: Do not touch the edge of well to avoid false results.
9. Repeat steps 4 and 5.
10. Incubate the plate in a 37±1°C incubator for 1 hour.
11. Repeat step 7 to wash the plate.
12. Develop the color by following HBeAg Detection steps 11-14.

Data Analysis

Calculation of Results

- HBeAg Detection

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

Example:

Sample No.	Absorbance
1	0.025
2	0.028
3	0.022

NCx = (0.025+0.028+0.022)/3 = 0.025

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

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

Example:

Sample No.	Absorbance
1	1.246
2	1.202

PCx = (1.246 + 1.202) /2 = 1.224

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

3. Calculation of the P - N Value

P - N = PCx – NCx

Example: P - N = 1.224 - 0.025 = 1.199

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

4. Calculation of the Cutoff Value

Cutoff Value = NCx + 0.06

Example: Cutoff Value = 0.025 +0.06 = 0.085

5. Calculation of the Retest Range

Retest Range = Cutoff Value ± 10%

Example: Cutoff Value = 0.085

Retest Range = (0.085 - 0.009) to (0.085 + 0.009) = 0.076 to 0.094

- Anti-HBe Detection

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

   Example:

Sample No.	Absorbance
1	0.888
2	0.915
3	0.909

   NCx =(0.888+0.915+0.909)/3= 0.904

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

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

   Example: Sample No. Absorbance

1	0.044
2	0.056

   PCx = (0.044+0.056)/2 = 0.050

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

3. Calculation of the N - P Value

   N - P = NCx –PCx

   Example: N - P = 0.904 - 0.050 = 0.854

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

4. Calculation of the Cutoff Value

   Cutoff Value = (NCx + PCx) /2

   Example: Cutoff Value = Cutoff Value = (0.904 + 0.050) / 2 = 0.477

5. Calculation of the Retest Range

   Retest Range = Cutoff Value ± 10%

   Example: Cutoff Value = 0.477

   Retest Range = (0.477 - 0.048) to (0.477 + 0.048) = 0.429 to 0.525

Result Interpretation

- HBeAg Detection

1. Specimens with absorbance values LESS than the Cutoff Value are Non-Reactive for HBeAg.
2. Specimens with absorbance values Greater than or Equal to the Cutoff Value are Reactive for HBeAg.
3. If the data is within the Retest Range the test must be repeated and interpreted as above.

- Anti-HBe Detection

1. Specimens with absorbance values Greater than the Cutoff Value are Non-Reactive for Anti-HBe.
2. Specimens with absorbance values Less than or Equal to the Cutoff Value are Reactive for Anti-HBe.
3. If the data is within the Retest Range, the test must be repeated and interpreted as above.

Resources

References

1. Incomplete inactivation of hepatitis B virus after heat treatment at +60℃ for 10 hours, J. Infect. Dis. 138:242-244.

Plate Layout

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