（**FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !**）

Human Hepatitis B Virus E (HBe) Antibody ELISA Kit

Catalog No: E-HD-E146

48T/96T

Version Number:	V1.0
Replace version:	V1.0
Revision Date:	2025.01.10

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help.

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.vetassay-elab.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Intended use

This ELISA kit applies to qualitative determination of Human HBeAb (Hepatitis B Virus E antibody) in serum and plasma.

Test principle

This ELISA kit uses the Competition-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with a Human HBeAg. Samples (or Control) and Horseradish Peroxidase conjugate antibody (HRP conjugate) are added to the micro ELISA plate wells, HBeAb will compete with HRP conjugate to combine with HBeAg coated on the ELISA plate. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human HBeAg and HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm (630 nm).

Kit components

Item	Specifications
ELISA Microtiter plate	96T：96 wells 48T：48 wells
Positive Control	96T：1.0 mL*1 vial 48T：0.5 mL*1 vial
Negative Control	96T：1.0 mL*1 vial 48T：0.5 mL*1 vial
HRP Conjugate	96T：6.0 mL*1 vial 48T：3.0 mL*1 vial
20×Concentrated Wash Buffer	96T：30 mL*1 vial 48T：20 mL*1 vial
Substrate Reagent A	96T：6.0 mL*1 vial 48T：3.0 mL*1 vial
Substrate Reagent B	96T：6.0 mL*1 vial 48T：3.0 mL*1 vial
Stop Solution	96T：6.0 mL*1 vial 48T：3.0 mL*1 vial
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: Unused HRP Conjugates, Positive Control, Negative Control, Concentrated Wash Buffer (20×), substrate A, substrate B, and Stop Solution should be stored in the original reagent bottle, all reagent bottle caps must be tightened to prevent evaporation and microbial pollution, stored at 2-

8 ℃ away from light.

The volume of reagents in partial shipments is a little more than the volume marked on the label, please use accurate measuring equipment instead of directly pouring into the vial(s).

Other materials required but not supplied

Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)

High-precision transferpettor, EP tubes and disposable pipette tips

37℃ Incubator or water bath

Deionized water

Absorbent paper

Loading slot for Wash Buffer

Notes

1. For research use only. Not for use in diagnostic procedures.
2. Please wear lab coats, eye protection and latex gloves for protection. Please perform the experiment following the national security protocols of biological laboratories, especially when detecting blood samples or other bodily fluids.
3. A freshly opened ELISA plate may appear a water-like substance, which is normal and will not have any impact on the experimental results. Return the unused wells to the foil pouch and store according to the conditions suggested in the above table.
4. The microplate reader should be able to be installed with a filter that can detect the wave length at 450±2 nm. The optical density should be within 0-3.5. Follow the Instructions of the Microplate Reader for set-up and preheat it for 15 min before OD measurement.
5. Do not mix or substitute reagents with those from other lots or sources.
6. Change pipette tips in between adding of each standard level, between sample adding and between reagent adding. Also, use separate reservoirs for each reagent.
7. The kit should not be used beyond the expiration date on the kit label.
8. The result judgment must be completed within 10 minutes of the termination of the reaction.
9. The test sample cannot be preserved with NaN_3.
10. This test kit should be considered as an infectious substance, please handle it according to the laboratory inspection procedures for infectious diseases.
11. Substrate A and substrate B should be added in sequentially, to avoid low color.
12. If there is flocculent sediment in the working wash buffer, it should be replaced immediately and the microplate washer should be cleaned.
13. Avoid operating in environments with volatile substances and hypochlorous acid disinfectants (such as 84 disinfectant).
14. The result judgment must be based on the Microplate reader. When reading the results, the bottom of the Micro ELISA Plate should be wiped dry and there should be no bubbles in the well. Do not touch the outer wall at the bottom of the hole, as fingerprints or scratches may affect the reading of the plate hole.

Storage and expiry date

Store unopened at 2 to 8℃. Do not freeze.

The unused wells should be immediately sealed in a self-sealing bag with desiccant, stored at 2-8 ℃ away from light

Expiry date: expiration date is on the box.

Sample preparation

1. Serum: Allow samples to clot for 1 hour at room temperature or overnight at 2-8℃ before centrifugation for 20 min at 1000×g at 2-8℃. Collect the supernatant to carry out the assay.
2. Plasma: Centrifuge samples for 15 min at 1000×g at 2-8℃ within 30 min of collection. Collect the supernatant to carry out the assay.
3. Serum and plasma samples should be stored at 2-8 ℃. For long time store, please store at -20 ℃ and avoid repeated freezing and thawing.
4. Samples containing anticoagulants such as EDTA, sodium heparin, or sodium citrate can be used in this test.
5. **Wash Buffer: The 20×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:19.

Assay procedure

Bring all reagents and samples to room temperature for about 30 min, restore to room temperature (25℃) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8℃.

1. Determine wells for **positive control**, **negative control**, **blank** and **sample**, each plate should set with 3 negative control holes, 2 positive control hole and 1 blank control hole (blank control wells may not be required when test under dual wavelength condition).
2. Add 50 μL positive control, negative control and sample into the appropriate wells.
3. Add 50μL **HRP Conjugate** into holes which have been added with positive control, negative control and sample, except for blank well, shake to mix well.
4. Cover the plate with the sealer provided in the kit. Incubate for 30 min at 37℃. Note: solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible.
5. Decant the solution from each well， add full file **wash buffer** into to each well. Soak for 30-60s and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 5 times. Note: a microplate washer can be used in this step and other wash steps. Make the tested strips in use immediately after the wash step. Do not allow wells to be dry.
6. Add 50 μL of **Substrate A** and **Substrate B** to each well, shake to mix well. Cover the plate with a new sealer. Incubate for about 15 min at 37 ℃. Preheat the Microplate Reader for about 15 min before OD measurement.
7. Add 50 μL of **Stop Solution** to each well, shake to mix well, the result should be read within 10 min.

Note: adding the stop solution should be done in the same order as the substrate solution.

8. Determine the optical density (OD value) of each well at once with a Microplate Reader set to 450 nm (It is recommended to use a dual-wavelength microplate reader, with a reference wavelength of 450 nm/ 600-650 nm). If it is necessary to subtract the blank, first set the blank well to zero, and then read the OD values of each hole.

Reference value

Calculation of reference value (Cut-Off): Cut-Off = Average OD value of negative control $\overline{NC}$ ×0.5.

Interpretation of test results

1. Normal range for negative controls: OD value of negative control ≥0.8 (If the OD value of one negative control was less than 0.8, the experiment should be discarded. If the OD value of two or more negative controls was less than 0.8, the experiment should be repeated).
2. Normal range for positive controls: OD value of positive control ≤0.1.
3. Positive judgement: OD value of sample ≤ Cut-Off, indicate HBeAb positive.
4. Negative judgement: OD value of sample ＞ Cut-Off, indicate HBeAb negative.

Limitations of test method

1. This test is only used as the qualitative detection of HBeAb in serum and plasma of human.
2. Test with Highly hemolytic samples, suspended fibrin or aggregate samples and samples preserved with NaN_3 may result in incorrect results.
3. Due to methodological limitations, a positive result must be judged in conjunction with other indicators.