Human Hepatitis B Virus E Antibody (HBeAb) ELISA Kit

Catalog No: abx257500

Size: 96T

Range: Qualitative

Sensitivity: Qualitative

Storage: Store at 2-8°C.

Application: The qualitative detection of HBeAb in Human serum, plasma and other biological fluids.

Principle of the Assay: This kit is based on competitive enzyme-linked immuno-sorbent assay technology. An antibody is pre-coated onto a 96-well plate. Controls, test samples, and HRP-conjugated reagent are then added to the wells and incubated. The HBeAb present in the sample competes with the HRP-conjugated reagent to bind to the pre-coated antibody. Unbound conjugates are removed using a wash buffer. TMB substrate is then added to trigger a colorimetric reaction. The OD values can then be measured using a microplate reader at 450 nm and the prescence of HBeAb can then be determined.

Kit Components
- Pre-coated 96-Well Microplate: 12 x 8
- 96-well Dilution Plate
- Wash Buffer: (20X) 30 ml
- Positive Control: 1 ml
- Negative Control: 1 ml
- Sample Diluent Buffer: 50 ml
- Detection Reagent: 7 ml
- TMB Substrate A: 7 ml
- TMB Substrate B: 7 ml
- Stop Solution: 7 ml
- Plate Sealer: 3
- Hermetic Bag: 1

Materials Required But Not Provided
- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Ice
- Microplate reader (wavelength: 450 nm)
- ELISA Shaker

For Reference Only

Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com

Protocol

A. Sample Preparation

Analyse immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles. Keep samples on ice during preparation. Equivalate samples to room temperature prior to analysis.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 1000 × g for 20 mins. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using EDTA, citrate or heparin as an anticoagulant. Centrifuge for 15 mins at 1000 × g, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples.
- **Other Biological Fluids:** Centrifuge at approximately 1000 × g for 20 mins to remove precipitate. Analyse immediately or aliquot and store at -20°C or -80°C.

Notes:

- Store frozen samples undiluted. Once ready to analyse, thaw samples and dilute.
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- NaN_3 cannot be used as a test sample preservative, since it inhibits HRP.
- If possible, prepare solid samples using sonication and/or homogenization, as lysis buffers may (on occasion) interfere with the kit's performance.
- If a sample is not indicated in the manuals applications, a preliminary experiment to determine the suitability of the kit will be required.

B. Reagent Preparation

Wash Buffer: Dilute the concentrated Wash buffer 20-fold with deionized water (i.e. add 30 ml of concentrated wash buffer into 570 ml of deionized water). If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

C. Assay Protocol

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure samples in duplicate.

1. Set 2 positive and 2 negative control, test sample and control (zero) wells on the pre-coated plate respectively, and record their positions.
2. Aliquot 50 µl of negative and 50 µl positive control into the set wells. Leave one well as the control (zero) blank well.
3. Aliquot 50 µl of appropriately diluted sample into the test sample wells. Gently tap the plate to mix, or use a microplate shaker.
4. Aliquot 50 µl of Detection Reagent to each well except the blank well.
5. Cover the plate with a plate sealer and incubate for 1 hr at 37°C.
6. Remove the cover, and discard the solution. Do not wash.
7. Remove the cover and discard the solution. Wash the plate 5 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (350 µl) using a multi-channel Pipette or autowasher (30 - 60 second soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting.*
8. Aliquot 50 µl of TMB Substrate A and 50 µl of TMB Substrate B into each well. Gently tap the plate to mix, or use a microplate shaker.
9. Cover the plate with a plate sealer and incubate for 15 minutes at 37°C in the dark.

For Reference Only

Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com

9. Remove the cover, and aliquot 50 µl of Stop Solution into each well. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
10. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the OD at 450 nm immediately.

Data Analysis:

- Mean OD of the Positive Control should be < Cut off .
- Mean OD of the Negative Control should be ≥ Cut off.

CUT OFF value = Negative Control × 0.3 (if the Mean OD of the Negative Control is < 0.05, calculate at 0.05; otherwise, calculate at the actual value)

- If the Positive Control value is > , and the Negative Control value is < , the test is valid, otherwise, the test is invalid.
- If OD of Samples ≥ CUT OFF, the test samples are considered negative.
- If OD of Samples < CUT OFF, the test samples are considered positive.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled. Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimised. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.
- Please note that this kit is optimised for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

Technical Support

For troubleshooting, frequently asked questions, and assistance, please visit:
https://www.abbexa.com/scientific-support/troubleshooting-and-faqs/elisa-kit-scientific-support or email us at support@abbexa.com.

For Reference Only

Abbexa LTD, Cambridge, UK · Phone: +44 (0) 1223 755950 · Fax: +44 (0) 1223 755951
Abbexa LLC, Houston, TX USA · Phone: +1 832 327 7413
Abbexa BV, Leiden, NL
Website: www.abbexa.com · Email: info@abbexa.com