

Human Anti-HBeAb ELISA Kit

Order details

CatNo: AHY-22

Size: 96T/48T

Product description

Hepatitis B virus (HBV) is an enveloped, double-stranded DNA virus belonging to the Hepadnaviridae family and is recognized as the major cause of blood transmitted hepatitis together with hepatitis C virus (HCV). Infection with HBV induces a spectrum of clinical manifestations ranging from mild, inapparent disease to fulminant hepatitis, severe chronic liver disease which in some cases can lead to cirrhosis and carcinoma of the liver. Anti-HBe ELISA is an enzyme-linked immunosorbent assay (ELISA) for qualitative detection of antibodies to hepatitis B virus e antigen (anti-HBe) in human serum or plasma. It is intended for use in clinical laboratories for diagnosis and management of patients related to infection with hepatitis B virus.

The hepatitis B e antigen (HBeAg), which is a product of the pre-C/C gene, has been found in the hepatocytes during proliferation of the hepatitis B virus. After proteolysis, the HBe protein is secreted into the serum in size varying from 16 kD to 20 kD. The e -antigen is associated with high degree of viral proliferation. HBeAg appears shortly after HBsAg and is detectable from few days to several weeks after infection. After treatment, in the recovery phase following acute hepatitis B, HBeAg - the first serological marker - becomes negative and is replaced by the corresponding antibody (anti-HBe). If HBeAg is considered a specific marker of infectivity, the presence of anti-HBe antibody in blood is recognized to be a clinical sign of recovery from the infection. Acute and persistent HBV infections can also occur without HBeAg being detectable. Demonstration of anti-HBe in these patients is an indication of the

presence HBV mutants. These may be associated with high, low, or non-detectable quantities of virus. For this, anti-HBe test is meaningful only in association with the HBeAg test for monitoring the course of an HBV infection.

Product description

Microplate	8x12/12x8-well strips per plate. Each well contains monoclonal antibodies reactive to HBeAg.
Negative Control	1ml,1 vial
Positive Control	1ml,1 vial
HRP-Conjugate	6.5ml,1 vial
Sample Diluent	7ml,1 vial
Substrate Solution A	7ml, 1 vial
Substrate Solution B	7ml, 1 vial
Stop Solution	7ml, 1 vial
Wash Buffer (20×)	30ml, 1 vial
Microtiterplate sealers	1 sheet
Plastic Sealable Bag	1 unit

Storage method: it can be stored at 2-8 °C for more than 6 months. Do not freeze it.

Steps

1. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve.
2. Set the strips needed in strip-holder and number sufficient number of wells including three Negative control (e.g. B1, C1, D1), two Positive control (e.g. E1, F1) and one Blank (e.g. A1, neither samples nor HRP-Conjugate should be added into the Blank well). If the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

3. Add Positive and Negative controls into their respective wells, add Sample Diluent into other reaction wells, then add sample, mix well.

Note: Use a separate disposable pipette tip for each specimen, Negative and Positive Control as to avoid cross-contamination.

4. Add HRP-Conjugate into each well except the Blank. Mix by tapping the plate gently.

5. Cover the plate with the plate cover and incubate at 37°C.

6. After the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Washing buffer. Each time allow the microwells to soak. After the final washing cycle, turn down the plate onto blotting paper or clean towel and tap it to remove any remainders.

7. Dispense Chromogen A and Chromogen B solution into each well including the Blank and mix by tapping the plate gently. Incubate the plate at 37°C avoiding light. The enzymatic reaction between the Chromogen A/B solutions and the HRP-Conjugate produces blue color in Positive control and HBeAg positive sample wells.

8. Using a multichannel pipette or manually, add Stop Solution into each well and mix gently. Intensive yellow color develops in Negative control and anti-HBe negative sample wells.

9. Calibrate the plate reader with the Blank well and read the absorbance at 450 nm. If a dual filter instrument is used, set the reference wavelength at 630 nm. Calculate the Cutoff value and evaluate the results. (Note: read the absorbance within 5 minutes after stopping the reaction.)

Note

1. The kit should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay. It is highly recommended that the solutions be used as soon as possible after rehydration.

2. When mixing or reconstituting protein solutions, always avoid foaming.

3. Do not mix or substitute reagents with those from other lots or sources.

4. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions.
5. Crystals could appear in the 20X wash solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
6. Keep TMB Substrate protected from light.
7. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.