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Human Anti-HBSAg ELISA Kit

Order details

CatNo: AHY-18

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 diseases, which in some cases can lead to cirrhosis and carcinoma of the liver.

HBsAg ELISA is an enzyme-linked immunosorbent assay (ELISA) for qualitative detection of HBsAg in human serum or plasma. It is intended for screening of blood donors and for diagnosing of patients related to infection with hepatitis B virus.

This HBsAg ELISA uses antibody "sandwich" ELISA method in which, polystyrene microwell strips are pre- coated with monoclonal antibodies specific to HBsAg. Patient's serum or plasma sample is added to the microwell together with a second antibody conjugated the enzyme horseradish peroxidase (the HRP-Conjugate) and directed against a different epitope of HBsAg. During incubation, the specific immunocomplex formed in case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum proteins and unbound HRP-conjugate, Chromogen solutions containing tetramethyl-benzidine (TMB) and urea peroxide are added to the wells. In presence of the antibody-antigen-antibody(HRP) "sandwich" immunocomplex, the colorless Chromogens are hydrolyzed by the bound HRP-conjugate to a blue-colored product. The blue color turns yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and it is proportional to the amount of antigen

captured in the wells, and to its amount in the sample respectively. Wells containing samples negative for HBsAg remain colorless.

Product description

Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibodies reactive to HBsAg (anti-HBs)
Negative Control	1ml,1 vial
Positive Control	1ml,1 vial
HRP-Conjugate	7ml,1 vial
Sample Diluent	30ml,1 vial
Substrate Solution A	8ml, 1 vial
Substrate Solution B	8ml, 1 vial
Stop Solution	8ml, 1 vial
Wash Buffer (20×)	50ml, 1 vial;
Microtiterplate sealers	2 sheets
Plastic Sealable Bag	1 unit

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

Steps

1. Allow all the reagents and samples to reach room temperature for at least 15-30 minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37℃ until crystals dissolve.

2. Mark three wells as Negative control (e.g. B1, C1, D1), two wells as 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. Cover the plate with the plate cover and incubate at 37℃.

5. 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.

6. Dispense Substrate Solution A and Substrate Solution B into each well including the Blank, and mix by tapping the plate gently. Incubate the plate at 37℃ avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue color in Positive control and HBsAg positive sample wells.

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

8. 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℃) 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. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of these substances.

7. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.