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Human Anti-HBSAb ELISA Kit

Order details

CatNo: AHY-19

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.

This anti-HBs ELISA kit is an enzyme linked-immunosorbent assay for in vitro qualitative detection of antibodies to hepatitis B virus surface antigen (anti-HBs) in human serum or plasma. It is intended for use in medical laboratories for diagnosis and management of patients related to infection with hepatitis B virus.

For detection of anti-HBs, this kit uses antigen "sandwich" ELISA method where polystyrene microwell strips are pre-coated with recombinant HBsAg. Patient's serum or plasma sample is added to the microwells together with a second HBsAg conjugated to Horseradish Peroxidase (HRP-Conjugate). In case of presence of anti-HBs in the sample, the pre-coated and conjugated antigens will be bound to the two variable domains of the antibody and during incubation, the specific immunocomplex formed is captured on the solid phase. After washing to remove sample serum proteins and unbound HRP-Conjugates, Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells. In presence of the antigen- antibody-antigen(HRP)

"sandwich" complex, 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 is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HBs remain colorless.

Product description

Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with purified HBsAg
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	6ml, 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 ℃ 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. 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. Add HRP-Conjugate into each well except for the Blank. Cover the plate with the plate cover and incubate at 37℃, and Washing as Step 5.

7. Stopping Reaction: Using a multichannel pipette or manually, add Stop Solution into each well. Intensive yellow color develops in Positive control and anti-HBs Positive 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. 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.