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Human Anti-HDV-Ag ELISA Kit

Order details

CatNo: AHY-27

Size: **96T**

Product description

Hepatitis D is a liver diseases caused by Hepatitis D virus (Delta agent) - a defective (36 nm-43 nm) enveloped RNA virus, which requires co-infection with Hepatitis B virus (HBV) for its replication. Transmitted percutaneously or sexually through contact with infected blood or blood products, HDV is associated with the most severe forms of chronic and acute hepatitis in many Hepatitis B-HBsAg positive patients. This kit is an enzyme-linked immunosorbent assay (ELISA) for qualitative determination of antigens to hepatitis D virus (HDV-Ag) in human serum or plasma (EDTA, heparin and Sodium Citrate). It is intended for use in clinical laboratories for diagnosis of patients related to infection with hepatitis D virus, and is good for the survey of epidemics.

The HDV-Ag ELISA employs the solid phase double antibody sandwich method. The patient's serum/plasma is added together with extraction solution after the polystyrene microwell strips are pre-coated with purified antibodies specific to HDV. If the HDV virus is present, the HDV particles are disrupted and what's captured in the wells is the specific HDV antigens. What is added next is Horseradish Peroxidase (HRP) which is conjugated with a secondary antibody. After washing, unbound conjugates are removed. Added to the wells after this are both the chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide. During this stage of a combined presence of antibody-antigen-antibody (HRP) sandwich immunocomplex, a bluecolored product appears, which is the result of colorless chromogens hydrolyzed by the bound HRP conjugate. After stopping the reaction

with sulfuric acid, the blue color turns yellow. The color intensity can be gauge proportionally to the amount of antigen in the sample. Colorless wells appear when samples are negative for HDV antigens.

Product description

Microplate	96 well polystyrene microplates (12 strips of 8 wells) coated with purified antibodies reactive to HDV
Negative Control	1ml,1 vial
Positive Control	1ml,1 vial
HRP-Conjugate	6ml,1 vial
Sample Diluent	6ml,1 vial
Substrate Solution A	6ml, 1 vial
Substrate Solution B	6ml, 1 vial
Stop Solution	6ml, 1 vial
Wash Buffer (20×)	50ml, 1 vial
Microtiterplate sealers	3 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. 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. Dispense TMB Solution A and TMB Solution B solution 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 TMB solutions and the HRP-Conjugate antibody produces blue color in Positive control and HDV-Ag positive sample wells.

7. Using a multichannel pipette or manually, add Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and HDV Ag 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℃ 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.