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Human Anti-HEV-Ab ELISA Kit

Order details

CatNo: AHY-33

Size: **96T**

Product description

Hepatitis E virus (HEV) is a non-enveloped, single- stranded RNA virus identified in 1999. Infection with HEV induces acute or sub-clinical liver diseases similar to hepatitis A.This HEV-Ab ELISA kit is an enzyme linked-immunosorbent assay for in vitro qualitative detection of total antibodies (IgG, IgM,etc.) to hepatitis E virus in human or animal serum or plasma. It is intended to be used as an aid in supplementary diagnosis to hepatitis E infection, prevalence studies among popula tions as well as in zoonosis related research on hepatitis E virus.

This HEV-Ab ELISA kit uses polystyrene microwell strips precoated with recombinant HEV antigens (HEV-Ag) corresponding to structural proteins ORF-2 of the native virus. Serum or plasma sample is added into the microwells. In case of presence of HEV-Ab in the sample, the pre-coated antigens will be bound to the antibody and during the first incubation step, the specific immunocomplex formed is captured on the solid phase. After washing to remove unbound sample, second recombinant HEV antigen conjugated to Horseradish Peroxidase (HRP) is added into the wells. During the second incubation step, this antigen will bind to the second variable domain of the HEV antibodies if they have been captured by HEV-antigen during the first incubation step. The unbound HRP conjugate is removed during washing and Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added into 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 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 HEV remain colorless.

Product description

Microplate	96 well polystyrene microplates coated with recombinant HEV antigens
Negative Control	0.5ml,1 vial
Positive Control	0.5ml,1 vial
HRP-Conjugate	12ml,1 vial
Sample Diluent	6ml,1 vial
Substrate Solution A	7ml, 1 vial
Substrate Solution B	7ml, 1 vial
Stop Solution	7ml, 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. 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 reagent to each well except the Blank, and mix by tapping the plate gently. Cover the plate with the plate cover and incubate at 37°C .At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash buffer. After the final washing cycle, turn the plate down onto blotting paper or clean towel, and tap it to remove any remainders.

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

8. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.