

Human Anti-HEV-Ag ELISA Kit

Order details

CatNo: AHY-32

Size: 96T/48T

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. HEV-Ag ELISA is an enzyme linked immunosorbent assay for the qualitative detection of Hepatitis E virus antigen in human serum and plasma samples.

HEV infections, endemic and frequently epidemic in developing countries, is seen also in developed countries in a sporadic form with or without a history of traveling to endemic area. The overall case-fatality is 0.5~3%, and much higher (15~25%) among pregnant women. A hypothesis that HEV infection is a zoonosis was presented in 1995. Then a swine HEV and later an avian HEV were identified and sequenced separately in 1997 and 2001. Since then, HEV infection include anti-HEV, viremia and feces excretion of HEV was seen in a wide variety of animals,i.e., swine, rodents, wild monkeys, deer, cow, goats, dogs and chicken in both the developing and developed countries.

Product description

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| Microplate | 1 plate(12x8/8x12 well strips per plate) |
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| Negative Control | 1ml,1 vial |
| Positive Control | 1ml,1 vial |
| HRP-Conjugate | 12ml,1 vial |
| Sample Diluent | 5ml,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 | 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. Cover the plate with the plate cover and incubate at 37°C.
5. At the end of the incubation. Remove and discard the plate cover. Add HRP Conjugate into each well except the blank. Cover the plate with 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. Add Chromogen A and Chromogen B solutions into each well including the Blank. Incubating the plate at 37°C avoiding light. The enzymatic reaction between the Chromogen solutions and the HRP Conjugate produces a blue colour in Positive controls and HEV antigen positive sample wells.
8. Using a multichannel pipette or manually, add stop solution into each well and mix gently, intensive yellow color develops in Positive control and HEV antigen positive 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.
8. The enzymatic activity of the HRP-conjugate might be affected from dust and reactive chemical and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such Substances.