Abvigen Inc
Tel: 1-816-388- 0112 Fax: 1- 888-616-0161
Email: info@abvigenus.com

Human Anti-HAV-IgM ELISA Kit

Order details

CatNo: AHY-17

Size: **96T/48T**

Product description

Hepatitis A is a self-limited disease and chronic stage or other complications are rare. The infection with HAV induces strong immunological response and elevated levels first of IgM and then IgG are detectable within a few days after the onset of the symptoms. The presence of anti – HAV IgM is an important serological marker for early detection and observation of the clinical manifestation of the disease. HAV-IgM ELISA is an enzyme-linked immunosorbent assay for qualitative determination of hepatitis A virus IgM-class antibodies in human serum or plasma samples.

This kit is a solid phase, two-step incubation, antibody capture ELISA assay in which, polystyrene microwell strips are precoated with antibodies directed to human immunoglobulin M proteins (anti- μ chain). The patient ' s serum/plasma sample is added and during the first incubation, any IgM antibodies will be captured in the wells. After washing out all the other components of the sample and in particular IgG antibodies, the specific HAV IgM captured on the solid phase is detected by the addition of HAV antigens conjugated to horseradish peroxidase (HRP-Conjugate). During the second incubation, the HRPconjugated antigens will specifically react only with the HAV IgM antibodies and after washing to remove unbound HRPconjugate, Chromogen solutions are added to the wells. In presence of the (anti-μ)-(HAV-IgM)-(antigen-HRP) 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 is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for HAV-IgM remain colorless.

Product description

Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with anti-IgM antibodies (anti- μ chain)
Negative Control	1ml,1 vial
Positive Control	1ml,1 vial
HRP-Conjugate	12ml,1 vial
Sample Diluent	12ml,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	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 for the Negative control, two for the Positive control and one Blank (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. Dispense Substrate Solution A and Substrate Solution B into each well including the Blank, and mix by tapping the plate gently. The enzymatic reaction between the TMB solutions and the HRP-Conjugate produces blue color in Positive control and HAV IgM positive sample wells.

8. 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.

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℃) 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.