Human Hepatitis A Virus IgM (HAV IgM) ELISA Kit

Catalog No.: abx257503

Size: 96 tests

Detection Range: Qualitative

Sensitivity: Qualitative

Storage: Store all of the kit components at 4°C.

Application: For qualitative detection of Human Hepatitis A Virus IgM in serum.

Principle of the Assay: This kit is based on the capture enzyme-linked immuno-sorbent assay principle. An anti-human IgM antibody is pre-coated onto a 96-well plate. Controls, test samples and HRP-conjugated reagent are added to the wells and incubated. Unbound conjugates are removed using wash buffer. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient bound HAV IgM will produce a blue colored product, which then changes to yellow after adding the acidic stop solution. The intensity of the yellow color is proportional to the HAV IgM amount bound on the plate. The Optical Density (OD) is measured spectrophotometrically at 450 nm in a microplate reader, from which the presence of HAV IgM can be determined.

Kit components

1. 96-well microplate
2. Sample Diluent Buffer: 12 ml
3. Wash Buffer: (20X) 50 ml
4. Positive Control: 1 ml
5. Negative Control: 1 ml
6. TMB Substrate A: 6 ml
7. TMB Substrate B: 6 ml
8. Detection Reagent: 12 ml
9. Stop Solution: 6 ml
10. Plate Sealer: 3
11. Hermetic Bag: 1

Materials required but not provided

1. Microplate reader (450 nm)
2. Double-distilled water
3. Pipette and pipette tips
4. 1.5 ml microcentrifuge tubes
5. Centrifuge
6. Vortex mixer
7. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 1000 × g for 20 mins. If precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C.

Note:

- **Analyze samples at an 11-fold (1/11) dilution (i.e. add 10 µl of sample to 100 µl of Sample Diluent Buffer, to obtain 110 µl of diluted sample).**
- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Store frozen samples undiluted. Once ready to analyze, thaw samples and dilute.
- NaN_3 cannot be used as a test sample preservative, since it inhibits HRP.
- Avoid samples containing suspended or fibrous protein. Avoid samples with significant hemolysis (hemoglobin > 10 g/L), hyperlipemia (triglycerides > 20 g/L), or high bilirubin content (bilirubin > 0.2 g/L).

2. Reagents

- **Wash Buffer:** Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e. add 20 ml of concentrated wash buffer into 380 ml of distilled water). If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

Note:

- Allow all reagents to equilibrate to room temperature before use.

B. Assay Procedure

Pre-heat the incubator and ensure it has reached a stable temperature before use.

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate.

1. Mark positions for 2 positive control wells, 3 negative control wells, 1 control (zero) well, and the sample wells on the pre-coated plate. **Samples must be tested in duplicate.** *When pipetting solution, add to the bottom of each well without touching the side walls. Pipette the samples up and down to mix before adding to the wells. Avoid foaming or bubbles.*
2. Aliquot 100 µl of negative control and 100 µl positive control into their respective wells. Leave the control (zero) well empty.
3. Aliquot 100 µl of appropriately diluted samples (10 µl of sample to 100 µl of Sample Diluent Buffer) into the test sample wells. Gently tap the plate to mix, or use a microplate shaker.
4. Cover the plate with a plate sealer and incubate for 30 mins at 37°C.
5. Remove the cover and discard the liquid. Wash the plate 5 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (350 µl) using a multi-channel pipette or autowasher (1-2 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.*
6. Aliquot 100 µl of Detection Reagent to each well (except the blank well).
7. Cover the plate with a plate sealer and incubate for 30 mins at 37°C.
8. Remove the cover, discard the liquid and repeat the wash step described above, 5 times.
9. Aliquot 50 µl of TMB Substrate A and 50 µl of TMB Substrate B into each well.
10. Cover the plate with the plate sealer and gently tap the plate to mix thoroughly. Incubate at 37°C for 15 mins. The incubation time is for reference only, the optimal time should be determined by end user. *Avoid exposure to light.*
11. Aliquot 50 µl of Stop Solution into each well. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to stop the reaction completely.
12. Measure the OD at 450 nm (or dual wavelength, at 450 / 630 nm) immediately. *Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles.*

C. Data Analysis

- Mean OD of the Positive Control should be > 0.80.
- Mean OD of the Negative Control should be < 0.08.
- Mean absorbance of the blank wells should be ≤ 0.08.
- CUT OFF value = Negative Control + 0.10 (if the Mean OD of the Negative Control is < 0.05, calculate at 0.05; otherwise, calculate at the actual value)

If OD of Samples < CUT OFF, the test samples are considered negative.

If OD of Samples ≥ CUT OFF, the test samples are considered positive.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimized. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.
- Please note that this kit is optimized for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.