## INTENDED USE

"Bio Tech Assay's HEV IgM is a Qualitative detection of human serum/ plasma of hepatitis E virus IgM.

## SUMMARY AND EXPLANATION

Hepatitis E is a liver infection caused by the hepatitis E virus (HEV), transmitted through the fecal-oral route. Typically, it causes acute and self-limiting infections with low death rates in Western countries. However, individuals with weakened immune systems, such as organ transplant recipients, are at risk of chronic hepatitis E with higher death rates. Pregnant women, especially in their third trimester, face a 20% mortality rate. A vaccine (HEV 239) is approved in China, and prevention relies on proper hygiene and sanitation practices.

## PRINCIPLE OF THE TEST

Capture method. Total duration of assay: 70 mins.

Hepatitis E virus (HEV) is a spherical RNA virus, 27-34nm in diameter, containing linear single-strand RNA. It causes an intestinal infectious disease with worldwide distribution, predominantly affecting young individuals, with a higher incidence in men than women.
The virus triggers the production of HEV-IgM and HEV-IgG antibodies. HEV-IgM appears shortly after infection, increases rapidly, then decreases and disappears in the late stages. In contrast, HEV-IgG emerges later and persists for an extended period.
This HEV IgM Detection Kit utilizes the capture ELISA principle to diagnose HEV infection in its early stages with high specificity. The process involves pre-coating purified anti-human IgM on microplates, which combines with HEV IgM in samples, forming antigen-antibody complexes with enzyme-labeled antigens that display a blue color in microplates, indicating positive results.
This kit is designed for specific detection of HEV IgM antibodies in human serum/plasma, offering a simple, fast, and reliable diagnostic tool.



| Sr. No | MATERIALS PROVIDED | 96 Tests |
|---|---|---|
| 1 | Positive Control: 1 vial (ready to use) | 0.2 ml |
| 2 | Negative Control: 1 vial (ready to 6use) | 0.2 ml |
| 3 | Enzyme conjugate: 1 bottle (ready to use) | 11 ml |
| 4 | Sample Diluent: 1 bottles (ready to use) | 11 ml |
| 5 | TMB Substrate: 1 bottle (ready to use) | 11ml |
| 6 | Stop Solution: 1 bottle (ready to use) | 6 ml |
| 7 | Wash concentrate 40X: 1 bottle | 25ml |
| 8 | Substrate 1 bottle | 0.2ml |
| 9 | Microwells coated | 12x8x1 |

**Additional Resources Needed**

- ELISA reader (450nm absorbance capability)
- Micro pipettes
- Disposable pipette tips
- Absorbent paper (or paper towel)
- Graph paper
- Distilled or deionized water

**Storage and Stability**

- Keep microwells sealed in a dry bag with desiccants.
- Reagents stable until kit expiration.
- Store kit at 2-8 °C.
- Avoid exposure to heat, direct sunlight, and strong light.

**Warnings and Precautions**

1. Research Use Only: Not intended for diagnostic procedures.
2. Handle as Biosafety Level 2 material.
3. Components contain human source materials, tested non-reactive for HIV, Hepatitis B, but handle with caution.
4. Follow test protocol strictly for optimal results.
5. Use pipettes, avoid mouth pipetting.
6. Refrain from smoking, eating, or drinking near specimens or reagents.
7. Use components from the same lot; avoid mixing.
8. Sodium azide in control sera and sample diluent may react with lead/copper plumbing; flush with water upon disposal.

**SPECIMEN COLLECTION AND HANDLING**

1. Separate serum from collected blood specimens.
2. Store specimens refrigerated at 2-8°C for up to one week, or frozen at -20°C or below for upto six months. To maintain sample quality, avoid multiple freeze-thaw cycles.

**REAGENT PREPARATION**

Dilute 25ml of 40X Wash Solution concentrate with 975ml of deionized water to create a 1000ml working solution, stable for 2 months at room temperature.

**Preparation**

To begin the assay, designate two wells as Negative Control (e.g., B1, C1), two wells as Positive Control (e.g., D1, E1), and one well as Blank (e.g., A1). Note that the Blank well should not contain samples or HRP-Conjugates. If using a dual-wavelength plate reader, the Blank well may be omitted.

**Sample Addition**

Add 100 µL of sample diluents to their respective wells, excluding the Blank. Then, add 10 µL of Positive Control, Negative Control, and specimen to their respective wells, mixing gently by tapping the plate. Use separate pipette tips for each specimen to prevent cross-contamination. After adding samples, the reagents in wells will turn blue from green.

**Incubation and Washing (First Round)**

Incubate the plate at 37°C for 30 minutes. After incubation, discard the plate cover and wash each well five times with diluted Wash Buffer, allowing 30-60 seconds of soaking time between washes. Remove excess liquid by tapping the plate onto blotting paper or a clean towel.

**Conjugate Addition**

Add 100 µL of conjugate to each well, excluding the Blank. Incubate at 37°C for 30 minutes.

**Incubation and Washing (Second Round)**

Repeat the washing step as before.

**Substrate Addition and Incubation**

Add 100 µL of substrate to each well, including the Blank. Incubate at room temperature for 10 minutes, avoiding light. The enzymatic reaction will produce a blue color in Positive Control and positive sample wells.

**Stopping the Reaction**

Stop the reaction by adding 50 µL of Stop Solution to each well and mixing gently. An intensive yellow color will develop in Positive Control and HSVI IgM-positive sample wells.

**CALCULATION OF RESULTS**

Finally, calibrate the plate reader with the Blank well and measure absorbance at 450 nm (with a 630 nm reference wavelength if using a dual-filter plate). Read absorbance within 10 minutes of stopping the reaction, then calculate the cut-off value and evaluate results.

**POSITIVE RESULT**

Samples with an absorbance level equal to or exceeding the cut-off value (C.O.) are deemed initially reactive, suggesting potential detection of HEV IgM antibodies via HEV ELISA. However, to confirm these findings, all initially reactive samples must undergo duplicate retesting using HEV ELISA. Only specimens that remain reactive after retesting can be confidently classified as positive for HEV IgM antibodies

**Negative Result**

Samples with absorbance below the cut-off value (C.O.) are considered negative for this assay, indicating no detection of HEV IgM antibodies via HEV ELISA. This suggests that the patient is likely not infected with Hepatitis E virus (HEV) and the blood unit does not contain HEV IgM. However, positive results require further confirmation through additional testing methods (e.g., PCR). A definitive clinical diagnosis should not rely solely on a single test result, but rather consider a comprehensive evaluation of clinical data, laboratory findings, and other relevant information.

**Limitations**

- Positive results must be confirmed with other available method and interpreted in conjunction with the patient clinical information.
- The reagent is qualitative, and cannot be used as a quantitative reagent.
- This reagent is only used for the detection of human serum /plasma samples.

**Performance Characteristics**

**Negative Specificity**: Our ELISA kits demonstrated 100% accuracy in identifying true negatives, with zero false positives detected in 20 national negative control samples.
**Positive Specificity:** Similarly, our kits showed 100% accuracy in identifying true positives, with no false negatives detected in 12 national positive control samples.
**Limit of Detection:** The kits successfully detected anti-HEV IgM in national limit quality control samples diluted 1:16, demonstrating excellent sensitivity.
**Precision:** The coefficient of variation (CV) remained below 15% when testing 10 wells with corresponding reference materials, ensuring consistent results.

Our ELISA test kit demonstrated exceptional clinical performance in a 1280-sample study, achieving a 99.5% negative agreement rate, 98.0% positive agreement rate, and 98.9% overall accuracy, showcasing its reliability in detecting anti-HEV IgM antibodies.



**References**

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