IgM Antibody to Hepatitis E Virus, HEV-IgM ELISA Kit

Prod. No.: DEIA074
Pkg. Size: 96T

INTENDED USE
HEV-IgM ELISA is an enzyme-linked immunosorbent assay for qualitative determination of IgM-class antibodies to hepatitis E virus in human serum or plasma samples. The assay is intended to be used in clinical laboratories for diagnosis and management of patients related to infection with hepatitis E virus.

PRINCIPLE OF THE TEST
This kit is a two-steps incubation, solid-phase antibody capture ELISA assay in which polystyrene microwell strips are pre-coated with antibodies directed to human immunoglobulin M proteins (anti-μ chain). The patient’s serum/plasma sample is added, and during the first incubation step, any IgM-class antibodies will be captured in the wells. After washing out all the other substances of the sample and in particular IgG-class antibodies, the specific HEV IgM captured on the solid phase is detected by the addition of recombinant HEV ORF2 antigen conjugated to the enzyme horseradish peroxidase (HRP-conjugate). During the second incubation, the HRP-conjugated antigens will specifically react only with HEV IgM antibodies. After washing to remove the unbound HRP-conjugate, chromogen solutions are added into the wells. In presence of (anti-μ) (anti-HEV-IgM) - (HEV Ag-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 amount of antibody in the sample respectively. Wells containing samples negative for HEV IgM remain colorless.

REAGENTS AND MATERIALS PROVIDED

Microplate: 96 well polystyrene microplates (12 strips of 8 wells) coated with anti-IgM antibodies (anti-μ chain);
Negative Control: 0.5 ml, 1 vial;
Positive Control: 0.5 ml, 1 vial;
HRP-Conjugate Antigen: 12ml, 1 vial;
Specimen Diluent: 12 ml, 1 vial;
TMB Solution A: 8 ml, 1 vial;
TMB Solution B: 8 ml, 1 vial;
TMB Stop Solution: 8 ml, 1 vial;
Wash Buffer (20X): 50 ml, 1 vial;
Microtiter plate sealers: 2 sheets;
Plastic Sealable Bag: 1 unit.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Validated microplate reader.
2. Eppendorf Tubes for dilution for samples and standards.
3. Deionized or distilled water.
4. Validated adjustable micropipettes, single and multi-channel.
5. Automatic microtiter plate washer or manual vacuum aspiration equipment.
6. 37°C incubator.

STORAGE
Unopened Kit: Store at 2 - 8°C. Do not use past kit expiration date.
Opened/Reconstituted Reagents: TMB Solution A; TMB Solution B; TMB Stop Solution; Wash Buffer; HRP-conjugate antibody
The above mentioned reagents should be stored for up to 1 month at 2 - 8°C.

Microplate Wells: Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8°C.

PRECAUTIONS
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. Also, use separate reservoirs for each reagent.
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.
7. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

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**SPECIMEN TREATMENT**

Centrifuge the serum, plasma or cell culture supernatant samples for 10 minutes at 1,000×g. Remove particulates and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

**ELISA PROTOCOL**

1. Allow the reagents and samples to reach room temperature for at least 15-30 minutes. 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. Dilute the stock Wash Buffer 1 to 20 with distilled or deionized water. Use only clean vessels to dilute the buffer.

2. Set the strips needed in strip-holder, and number sufficient number of wells including three for the Negative control (e.g. B1, C1, D1), two for the Positive control (e.g. E1, F1) and one Blank (e.g. A1, neither samples nor HRP-Conjugate antibody 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 100 µl Specimen Diluent into each well. Add 10 µl of Positive control, Negative control, and specimen into their respective wells. Note: Use a separate disposal pipette tip for each specimen, Negative and Positive Control as to avoid cross-contamination. Cover the plate with the plate cover and incubate for 30 minutes at 37°C. At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash buffer. Each time, allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the plate down onto blotting paper or clean towel, and tap it to remove any remainders.

4. Add 100µl 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 for 30 minutes at 37°C. At the end of the incubation, remove and discard the plate cover. Wash each well 5 times with diluted Wash buffer. Each time, allow the microwells to soak for 30-60 seconds. After the final washing cycle, turn the plate down onto blotting paper or clean towel, and tap it to remove any remainders.

5. Dispense 50 µl of TMB Solution A and 50 µl TMB Solution B solution into each well including the Blank, and mix by tapping the plate gently. Incubate the plate at 37°C for 15 minutes avoiding light. The enzymatic reaction between the TMB solutions and the HRP-Conjugate antibody produces blue color in Positive control and HEV IgM positive sample wells.

6. Using a multichannel pipette or manually, add 50 µl Stop Solution into each well and mix gently. Intensive yellow color develops in Positive control and HEV IgM positive sample wells.

7. 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 Cut-off value and evaluate the results. (Note: read the absorbance within 5 minutes after stopping the reaction).

**INTERPRETATION OF RESULTS AND QUALITY CONTROL**

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by relating each sample’s optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and controls. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and controls.

**1. Calculation of Cut-off value:**

\[
\text{Cut-off value (C.O.)} = \ast N_c + 0.26
\]

\[
\ast N_c = \text{the mean absorbance value for three negative controls.}
\]

**Example:**

<table>
<thead>
<tr>
<th>Well No</th>
<th>B1</th>
<th>C1</th>
<th>D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative controls OD value</td>
<td>0.012</td>
<td>0.010</td>
<td>0.011</td>
</tr>
<tr>
<td>Well No</td>
<td>E1</td>
<td>F1</td>
<td></td>
</tr>
<tr>
<td>Positive controls OD value</td>
<td>2.363</td>
<td>2.436</td>
<td></td>
</tr>
</tbody>
</table>

All control values are within the stated quality control range.

2. Calculation of \(N_c=(0.012+0.010+0.011)/3=0.011\)

3. Calculation of Cut-off value: Cut-off (C.O.) = 0.011 + 0.26 = 0.271

If one of the Negative control values does not meet the Quality control range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

**2. Quality control range:**

The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.
1. The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm, or at 450nm after blanking.

2. The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.

3. The OD value of the Blank well, which contains only Chromogen and Stop solution, is less than 0.080 at 450 nm.

3. Interpretations of the results:

(S = the individual optical density (OD) of each specimen)

**Negative Results (S/C.O.<1):** Samples giving absorbance less than the Cut-off value are negative for this assay, which indicates that no HEV IgM-class antibodies have been detected with HEV-IgM ELISA kit, therefore there are no serological indications for current infection with HEV.

**Positive Results (S/C.O.≥1):** Samples giving an absorbance which is equal to, or greater than the Cut-off value are considered initially reactive, which indicates that IgM-class antibodies to hepatitis E virus have probably been detected using this HEV IgM ELISA kit. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for IgM-class antibodies to HEV and therefore the patient is probably infected with hepatitis E virus.

**Borderline (S / C.O. = 0.9-1.1):** Samples with absorbance to Cut-off ratio between 0.9 and 1.1 are considered borderline and retesting of these specimens in duplicates is required to confirm the initial results. If, after retesting of the initially reactive samples, both wells are negative results (A/C.O.<0.9), these samples should be considered as non-repeatable positive (or false positive) and recorded as negative. As with many very sensitive ELISA assays, false positive results can occur due to the several reasons, most of which are connected with, but not limited to, inadequate washing step. If after retesting, one or both wells are positive results, the final result from this ELISA test should be recorded as repeatedly reactive. Repeatedly reactive specimens could be considered positive for HEV IgM antibodies. After retesting in duplicates, samples with values close to the Cut-off value should be interpreted with caution and considered as "borderline" zone sample, or uninterpretable for the time of testing.

**Limitations**

Positive results must be confirmed with another available method and interpreted in conjunction with the patient clinical information.

1. The assay is designed to achieve very high performance characteristics of sensitivity and specificity and the "sandwich model" minimizes the unspecific reactions due to interference with unknown matters in specimen (false positive results).

2. Antibodies may be undetectable during the early stage of the disease and in some immunosuppressed individuals. Therefore, negative results obtained with HEV-gM ELISA are only indication that the sample does not contain detectable level of HEV IgM-class antibodies and any negative result should not be considered as conclusive evidence that the individual is not infected with HEV.

3. The most common sources for mistakes are: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add specimens or reagents, improper operation with the laboratory equipment, timing errors, the use of highly hemolytic specimens or specimens containing fibrin, incompletely clotted serum specimens.

4. The prevalence of the marker will affect the assay’s predictive values.

5. HEV-IgM ELISA is a qualitative assay and the results cannot be used to measure antibodies concentrations.

**Indications of Instability or Deterioration of the Reagents**

1. Values of the Positive or Negative controls, which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones, or contact our technical support for further assistance.

2. If after mixing of the TMB Solution A and B into the wells, the color of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

**References**


