Hepatitis E virus IgM (Human) ELISA Kit

Catalog Number KA1009

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

This Hepatitis E virus IgM (Human) ELISA Kit is to be used for the in vitro detection of IgM antibody to Hepatitis E Virus (HEV) in human serum or plasma. This kit is intended for LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

Background

Hepatitis resulting from infection with viruses other than Hepatitis A Virus (HAV) and Hepatitis B (HBV) virus was previously referred to as non-A, non-B hepatitis. The first characterised non-A, non-B hepatitis agent was that responsible for parentally transmitted non-A, non-B hepatitis, or what is now called Hepatitis C Virus. This was followed by the cloning of a portion of the fecal-orally-transmitted agent, the Hepatitis E Virus (HEV). Hepatitis E Virus has been referred to as enterically transmitted non-A, non-B hepatitis.

Epidemics of enterically transmitted Hepatitis E Virus have been recognised worldwide but occur principally in developing countries. They have been reported in Southeast Asia, central Asia, Africa, Mexico, and Central America. In these areas, contaminated water has been implicated as the principal vehicle of virus transmission. Although HEV and HAV are transmitted in a similar manner, there are major differences in the clinical, pathological, and epidemiological courses of these two viruses. In particular, the mortality rate for HEV infection is 1 to 2%, or approximately 10-fold greater than that seen for HAV. Infection with HEV is particularly fatal for pregnant women, for whom the mortality rate can be as high as 10 to 20%.

Principle of the Assay

This Hepatitis E virus IgM (Human) ELISA Kit employs a technique called a qualitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a mixture of synthetic and recombinant HEV antigens that correspond to the structure regions of HEV. Samples are diluted 1:100 with HEV buffer and added to the microtiter plate wells and incubated. HEV specific antibodies if present will bind and become immobilized by the antigen pre-coated on the wells. The microtiter plate wells are thoroughly washed to remove unbound components of the sample. A standardized preparation of horseradish peroxidase (HRP) conjugated goat anti-human IgM antibody is added to each well to “sandwich” the HEV antibody immobilized during the first incubation. The microtiter plate then undergoes a second incubation. The wells are thoroughly washed to remove all unbound HRP conjugate and a TMB (3,3',5,5' tetramethyl-benzidene) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain HEV antibody and enzyme-conjugate will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm. Samples with O.D. values greater than or equal

to the Cut-off Value are considered reactive by the criteria of this Hepatitis E virus IgM (Human) ELISA Kit. This high specificity HEV-IgM assay uses 1:100 diluted samples to reduce the cross-reactivity causing factors in blood sample, while reduced antibody signal is compensated by a much concentrated anti-IgM conjugate. When compared with conventional methods, this kit has consistently demonstrated a higher specificity, and improved Signal to Cut-off (S/CO) ratio for the positive samples.

General Information

Materials Supplied

List of component

Component	Amount
Mcrotiter Plate: Precoated with mixture of synthetic HEV polypeptides.	96 wells
Conjugate: Horseradish peroxidase conjugated goat anti-human IgM antibody. Ready to use.	12 mL
Non-Reactive Control: Inactivated normal human serum diluted in sample diluent, to be used without dilution.	1 mL
Reactive Control: Inactivated human serum, to be used without dilution.	1 mL
Sample Diluent: Buffered solution with animal serum and preservative.	60 mL
Wash Buffer (20X): 20-fold concentrated solution of buffered surfactant.	60 mL
Substrate A: Buffered solution with H_2O_2.	10 mL
Substrate B: Buffered solution with TMB.	10 mL
Stop Solution: 2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!	14 mL

Storage Instruction

All reagents provided are stored at 2-8℃. Refer to the expiration date on the label.

Materials Required but Not Supplied

- ✓ Single or multi-channel precision pipettes with disposable tips: 5-100 µL and 50-200 µL for running the assay.
- ✓ Pipettes: 1 mL, 5 mL, 10 mL and 25 mL for reagent preparation.
- ✓ Multi-channel pipette reservoir or equivalent reagent container.
- ✓ Test tubes and racks.
- ✓ Polypropylene tubes or containers (25 mL).
- ✓ Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
- ✓ Incubator (37 ± 2℃)
- ✓ Microtiter plate reader (450 nm ± 2 nm)
- ✓ Automatic microtiter plate washer or squirt bottle
- ✓ Sodium hypochlorite solution, 5.25% (household liquid bleach).
- ✓ Deionized or distilled water
- ✓ Plastic plate cover.
- ✓ Disposable gloves.
- ✓ Absorbent paper.

✓ Test tubes for diluting assay samples.

Precautions for Use

✓ Precautions

- Do not substitute reagents from one package lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
- Allow kit reagents and materials to reach room temperature (20-25℃) before use. Do not use water baths to thaw samples or reagents.
- Do not use reagents beyond their expiration date.
- Use only deionized or distilled water to dilute reagents.
- Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8℃ in their pouch with the desiccant provided.
- Use fresh disposable pipette tips for each transfer to avoid contamination.
- Do not mix acid and sodium hypochlorite solutions.
- Human serum, plasma and the controls in kit should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
- All samples should be disposed of in a manner initially.
  Solid Waste: Autoclave 60 min. at 121℃.
  Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
- Substrate Solution is easily contaminated. If bluish prior to use, do not use.
- Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
- If Wash Buffer (20X) is stored at a lower temperature (2-5℃), crystals may form which must be dissolved by warming to 37℃ prior to use.
- If Sample Diluent is stored at a lower temperature (2-8℃), pellet may form which must be dissolved by warming to room temperature prior to use.
- Although this ELISA Kit is just for research use only, the end user should follow the HEV and related regulation issued by local authority to treat the samples and all materials of this Kit. The precautions 8 and 9 are just the suggestion for your treatment initially.

✓ Limitations of the procedure

- FOR LABORATORY RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Repeatedly reactive results from Hepatitis E virus IgM (Human) ELISA Kit are presumptive evidences of IgM antibodies to HEV in the specimen. A Non-reactive result from Hepatitis E virus IgM (Human) ELISA Kit indicates the likely absence of detectable IgM antibodies to HEV in the specimen. A negative result

does not exclude the possibility of exposure to or infection of HEV, because HEV IgM antibody appears early during clinical process but disappears rapidly over a few months while HEC IgG antibody appears a few days later and persists at least a few years.

Assay Protocol

Reagent Preparation

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25℃). Prepare the following reagents as indicated below.

1. Non-Reactive Control, and Reactive Control: Supplied in pre-diluted form. DO NOT DILUTE
2. Wash Buffer: Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly by gently swirling. Avoid foaming. If a smaller volume of Wash Buffer is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer is stable for 1 month at 2-8℃. Mix well before use.
3. Samples: Before assay is started, aliquot 500 µL of assay buffer to test tubes and mix gently and thoroughly with 5 µL of sample to obtain 1:100 diluted samples. The diluted samples should be used immediately.
4. Substrate Solution: Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (ml)	Substrate B (ml)	Substrate Solution (ml)
16 wells	1.5	1.5	3.0
32 wells	3.0	3.0	6.0
48 wells	4.0	4.0	8.0
64 wells	5.0	5.0	10.0
80 wells	6.0	6.0	12.0
96 wells	7.0	7.0	14.0

Sample Preparation

- ✓ Serum: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4℃) and serum extracted. This kit is for use with serum samples without additives only.
- ✓ Plasma: Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, after collection there must be quick separation of plasma with less than 30 minutes on ice. Centrifuge for 10 minutes (4℃) to remove any particulates. This ELISA kit is not affected by haemolysis of specimens. No adverse effects have been noted in the presence of anti-coagulants, sodium citrate, EDTA, or heparin.
- ✓ *Note:*
- *Avoid grossly hemolytic, lipidic or turbid samples.*
- *Serum samples to be used within 24-48 hours may be stored at 2-8℃, otherwise samples must stored at*

-20℃ to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.

- *When performing the assay slowly bring samples to room temperature.*
- *It is recommended that all samples be assayed in duplicate.*
- *DO NOT USE HEAT-TREATED SPECIMENS.*

Assay Procedure

1. Prepare 1:100 diluted samples and Wash Buffer before starting assay procedure (see Preparation of Reagents). It is recommended that the table provided be used as a reference for adding Controls and Samples to the Microtiter Plate. Use sample diluent as blank control.

Wells	Contents	Wells	Contents
A1, B1	Blank Control (sample diluent)	F1, G1	Reactive Control (RC)
C1, D1, E1	Non-Reactive Control (NRC)	H1.....	Samples

2. Add 100 µL of Blank Control, Non-Reactive Control, and Reactive Control to the appropriate wells of the Microtiter Plate. DO NOT DILUTE.
3. Add 100 µL of 1:100 diluted samples to wells designated for each sample.
4. Cover and incubate the Microtiter Plate for 30 minutes at 37° C ± 2℃.
5. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer, then aspirate contents of the plate into a sink or proper waste container.

   Repeat this procedure four more times for a total of FIVE washes. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note: Hold the sides of the plate frame firmly when washing the late to assure that all strips remain securely in frame*.

   Automated Washing: Aspirate all wells, then wash plates FIVE times using Wash Buffer. Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. It is recommended that the washer be set for a soaking time of 10 seconds or shaking time or 5 seconds between washes.
6. Add 100 µL Conjugate to each well. Cover and incubate plate for 30 minutes at 37° C ± 2℃.
7. Prepare Substrate Solution (see Preparation of Reagents) no more than 15 minutes before end of second incubation.
8. Repeat wash procedure as described in Step 5
9. Add 100 µL Substrate Solution to each well. Cover and incubate Microtiter Plate for 10 minutes at 37° C ± 2℃.
10. Add 100 µL Stop Solution to each well. Mix well.
11. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

✓ Quality Control

1. For each plate with each run of samples the Blank and Reactive Control should be assayed in duplicate and the Non-Reactive Control in triplicate.
2. Blank Control values must have an O.D. of ≤ 0.100.
3. Non-Reactive Control values must have an O.D.≤ 0.150 after subtracting the Blank Control value.
4. The Reactive Control values must have an O.D. value ≥ 1.0 after subtracting the Blank Control value.
5. Two of the Reactive Control values must not deviate by > 30% from the control mean. If this occurs the run is not valid and the assay procedure must be repeated.
6. For the assay to be valid, the mean O.D. difference between Reactive Control and Non-Reactive Control must be ≥ 1.0 (RC - NRC ≥ 1.0). If not, poor technique must be suspected and the assay must be repeated. Reagent deterioration may be suspected if the RC - NRC is consistently low.

Data Analysis

Calculation of Results

1. All O.D. values for Controls and Samples are subtracted by the O.D. value of the Blank Control before results interpretation. If more than one plate is being assayed at the same time, each plate must be calculated and interpreted separately.
2. The presence or absence of antibody to HEV is determined by relating the O.D. of the samples to the CUT-OFF value.

   CUT-OFF value = Mean O.D. of Non-Reactive Control ($\overline{NRC}$) + 0.20.

   If the Avg. OD of Non-Reactive Controls is slightly lower than that of the Blank Control (within 0.01), the experiment is still valid. In the situation in that the value of avg. OD of Non-reactive Control subtracted by that of Blank Control is less than 0.2:

   CUT-OFF value= 0.02 + 0.20= 0.22

✓ Calculation of Blank Control Mean O.D. ($\overline{BC}$) Example:

Well No.	O.D
A1	0.022
B1	0.024
Total	0.046
Mean	0.046/2 = 0.023 ($\overline{BC}$)

✓ Calculation of Non-Reactive Control Mean O.D. ($\overline{NRC}$) Example:

Well No.	O.D	BC subtracted
C1	0.060	0.037
D1	0.062	0.039
E1	0.059	0.036
Total		0.112
Mean		0.112/3 = 0.037 ($\overline{NRC}$)

✓ Calculation of Reactive Control Mean O.D. ($\overline{RC}$) Example:

Well No.	O.D	BC subtracted
F1	2.207	2.184
G1	2.198	2.175
Total		4.359
Mean		4.359/2 = 2.180 ($\overline{RC}$)

✓ Calculation of Reactive Control Mean - Non-Reactive Control Mean ($\overline{RC}$ - $\overline{NRC}$) Example:

$\overline{RC}$	2.180
$\overline{NRC}$	0.037
$\overline{RC}$ - $\overline{NRC}$	2.180 - 0.037 = 2.143

✓ Calculation of CUT-OFF Value Example:

CUT-OFF value	$\overline{NRC}$ + 0.200
$\overline{NRC}$	0.037
CUT-OFF value	0.037 + 0.200 = 0.237

✓ Interpretation of Results

1. Samples with O.D values LESS THAN the CUT-OFF value are considered NON- REACTIVE by the criteria of this HEV IgM Antibody ELISA Kit.
2. Samples with O.D values GREATER THAN or EQUAL to the CUT-OFF value are considered INITIALLY REACTIVE for antibodies to HEV by the criteria of the HEV IgM Antibody ELISA Kit and should be re-tested in duplicate before interpretation.

Performance Characteristics

The mean signal to cut-off ratio (S/CO) is defined as the mean sample O.D. divided by the calculated Cut-off value. The assay precision is determined by intra-assay and inter-assay standard deviation (S.D.) and the coefficient of variation (%CV).

✓ Intra-assay precision

To determine within-run precision, four different samples were assayed by replicates of twelve in one assay. The within assay derivation for each sample was calculated and listed as follow:

Sample	1	2	3	4	Sample	RC	NRC
Sample replication	12	12	12	12	Sample replication	2	2
S/CO	2.758	7.23	2.838	7.272	Mean CO	2.578	0.0705
S.D.	0.024	0.030	0.018	0.018	S.D.	0.020	0.004
%CV	3.21	1.53	2.34	0.9	%CV	0.77	5.0

✓ Inter-assay precision

To determine between-run precision, four different samples were assayed by replicates on nine different assays. The inter-assay coefficient of variation of the Signal to Cut-Off ratio (S/CO) for each sample was calculated and listed as follow:

Sample	1	2	3	4
Assay replication	9	9	9	9
Mean S/CO	2.587	6.757	2.714	7.043
%CV of S/CO	6.0	6.3	8.0	6.3

✓ Specificity and Sensitivity

The specificity of Hepatitis E virus IgM (Human) ELISA Kit was estimated based on HEV-IgM detection of blood samples from a normal donor population.

Sample Type	Number tested	Number negative	Specificity
Normal USA donor	102	100	98%

The improved sensitivity and specificity of this H-HEV-IgM were demonstrated by testing randomly selected HEV-IgG positive sera collected from patients during an HEV outbreak in China. Results consistently proved that the Hepatitis E virus IgM (Human) ELISA Kit improved sensitivity, while the false positive results in normal population is significantly reduced.

The following example showed that Hepatitis E virus IgM (Human) ELISA Kit increased S/CO ratio in serum samples (GP-GP23) collected from patients (including convalescent samples) and decreased S/CO ratio in samples (N49, N63, N121) from normal donor.

	High Specificity HEV-IgM			Conventional Method		
Sample	Mean OD	S/CO Ratio		Mean OD	S/CO Ratio	
GP1	0.45	1.76	+	0.446	1.27	+
GP2	0.922	3.61	+	0.889	2.53	+
GP3	0.216	0.85	-	0.298	0.85	-
GP5	0.295	1.15	+	0.428	1.22	+
GP6	0.588	2.3	+	0.663	1.89	+
GP7	0.593	2.32	+	0.509	1.45	+
GP9	0.372	1.46	+	0.298	0.85	-
GP10	1.068	4.18	+	0.871	2.48	+
GP11	2.465	9.65	+	1.386	3.95	+
GP12	1.029	4.03	+	0.958	2.73	+
GP20	0.509	1.99	+	0.826	2.35	+
GP21	0.775	3.03	+	0.566	1.61	+
GP22	2.357	9.23	+	1.416	4.03	+
GP23	1.621	6.34	+	1.174	3.34	+
N49	0.142	0.56	-	0.327	0.93	-
N63	0.161	0.63	-	0.295	0.84	-
N121	0.128	0.5	-	0.346	0.98	-
BC	0.056	0.22		0.051	0.15	
PC	2.584	10.1		1.172	3.34	
NRC	0.028			0.033		
Cut-off	NRC+0.2=0.256			NRC+0.03=0.351		

Resources

Reference

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Plate Layout

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