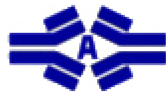
HEV IgG Antibody ELISA Kit

For the Detection of IgG Antibody to Hepatitis E Virus (HEV) in Human Serum or Plasma.

Catalogue Number: EL10002

96 tests

FOR LABORATORY RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES



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INTENDED USE

This HEV IgG Antibody ELISA is to be used for the *in vitro* detection of IgG 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.

INTRODUCTION

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. Increasingly, hepatitis E is found in developed countries. Domestic animals have been reported as a reservoir for this virus. HEV infection in most cases is self-limiting and could be asymptomatic sometimes.

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%.

This HEV Antibody ELISA is an immunoassay, which employs synthetic and recombinant HEV antigens, for the detection of antibodies to HEV in human serum or plasma. These antigens, which correspond to the structure regions of HEV, constitute the solid phase antigenic adsorbent that binds to antibodies that recognises the viral antigen. The detection of anti-HEV IgG in human blood indicates current viral infection or previous expose to this virus.

Samples with O.D. values greater than or equal to the Cut-off value are defined as initially reactive. Initially reactive specimens are to be re-tested in duplicate. Samples, which do not react in either of the duplicate, are considered non-reactive for antibodies to HEV. Samples, which are reactive in either of the duplicates tests, are considered repeatedly reactive.

PRINCIPLE OF THE ASSAY

This HEV Antibody enzyme linked immunosorbent assay (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 or controls are 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 IgG 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 HEV Antibody ELISA Kit.

REAGENTS PROVIDED

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

96 tests

1. **MICROTITER PLATE** (Part EL02-1) ***96 wells***
   Pre-coated with HEV antigens

2. **CONJUGATE** (Part EL02-2) ***12 mL***
   Horseradish peroxidase conjugated goat anti-human IgG antibody. Ready to use.

3. **NON-REACTIVE CONTROL** (Part EL02-3) ***1 mL***
   Inactivated normal human serum diluted in sample diluent.

4. **REACTIVE CONTROL** (Part EL02-4) ***1 mL***
   Inactivated human serum.

5. **SAMPLE DILUENT** (Part EL02-5) ***12 mL***
   Buffered solution with animal serum, preservative and color indicator.

6. **WASH BUFFER (20X)** (Part 30005) ***60 mL***
   20-fold concentrated solution of buffered surfactant.

7. **SUBSTRATE A** (Part EL02-6) ***10 mL***
   Buffered solution with H_2O_2.

8. **SUBSTRATE B** (Part 30007) ***10 mL***
   Buffered solution with TMB.

9. **STOP SOLUTION** (Part 30008) ***14 mL***
   2N sulphuric acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

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

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Controls, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum, plasma and the Controls in the 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.
9. All materials should be disposed of in a manner initially.
    Solid Waste: Autoclave 60 min. at 121°C.
    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.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.
13. If Sample Diluent is stored at lower temperature (2-8°C), pellet may form which must be

dissolved by warming to room temperature prior to use.

SAMPLE PREPARATION

COLLECTION, HANDLING ,AND STORAGE

a) **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°C) and serum extracted.
b) **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°C) to remove any particulates. *This HEV Antibody 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*.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum, plasma samples to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must be stored at -20°C 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.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). 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°C. Mix well before use.

3. 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.

Wells 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

ASSAY PROCEDURE

1. Prepare 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)	**E1, F1, G1**	Reactive Control (RC)
C1, D1	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. Prepare a 1:20 dilution of each sample by, first pipetting 95 µL of Sample Diluent into the appropriate Microtiter Plate wells, then adding 5 µL of sample. Mix well. The color of the sample diluent will change to blue after adding the sample.

4. Cover and incubate the Microtiter Plate for **30 minutes at 37°C**.

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 plate 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 of 5 seconds between washes.*

5. Add 100 μL Conjugate Solution to each well. Cover and incubate plate for **20 minutes at 37°C**.

6. Prepare Substrate Solution (see Preparation of Reagents) no more than 15 minutes before end of second incubation.

7. Repeat wash procedure as described in Step 4.

8. Add 100 μL Substrate Solution to each well. Cover and incubate Microtiter Plate for **15 minutes at 37°C**.

9. Add 100 μL Stop Solution to each well. Mix well.

10. Read the Optical Density (O.D.) at 450nm using a microtiter plate reader within 30 minutes.

QUALITY CONTROL

1. For each plate with each run of samples the Blank and Non-Reactive Control should be assayed in duplicate and the Reactive Control in triplicate.

2. The mean O.D. of the Blank Control should be less than or close to the mean O.D. of the NRC. Otherwise, factors such as an edge effect or insufficient washing may have contributed to the discrepancy.

3. The Reactive Control values must have at least two of the three with an O.D. value ≥ 0.6 *after subtracting the Blank Control value.*

4. Two or more 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.

5. For the assay to be valid, the mean O.D. difference between Reactive Control and Non-Reactive Control must be ≥ 0.500 (RC - NRC ≥ 0.500). 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.

RESULTS

1. 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 (NRC) + 0.20

CALCULATION OF RESULTS

1. **Calculation of Blank Control Mean O.D. (BC) (*Not required in Calculations)**

Example:	***Well No.***	***O.D***
	A1	0.044
	B1	0.057
	Total	0.101
	Mean	0.050 (BC)

2. **Calculation of Non-Reactive Control Mean O.D. (NRC)**

Example:	***Well No.***	***O.D.***
	C1	0.061
	D1	0.060
	Total	0.121
	Mean	0.060

3. **Calculation of Reactive Control Mean O.D. (RC)**

Example:	**Well No.**	***O.D.***
	E1	2.006
	F1	2.033
	G1	2.020
	Total	6.059
	Mean	2.020

4. Calculation of CUT-OFF Value

CUT-OFF value = NRC + 0.2

Example:	NRC	=	0.060
	CUT-OFF value	=	0.060 + 0.2
		=	0.260

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 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 Antibody ELISA Kit and should be re-tested in duplicate before interpretation.

3. Samples that are found reactive on re-testing are interpreted to be REPEATEDLY REACTIVE for antibodies to HEV by the criteria of this HEV Antibody ELISA Kit.

4. Initially reactive samples, which are found to be non-reactive on re-testing, are considered NEGATIVE by the criteria of this HEV Antibody ELISA Kit.

PERFORMANCE CHARACTERISTICS

The performance characterization was done with the samples collected during a Hepatitis E outbreak in China. The performance data are intended to use as a reference for research only. For clinical diagnostic use, only those assays that have been approved by local health administration should be considered.

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 intra-assay and inter-assay standard deviation (S.D) and the coefficient of variation (%CV) were calculated.

1. **Intra-assay precision:**
   To determine within-run precision, four different samples were assayed by replicates of twelve in one assay.

Sample	1	2	3	4	**Control**	RC	NRC
N	12	12	12	12	**N**	12	12
S/CO	2.49	2.15	2.90	4.44	**Mean**	1.471	0.041
S.D.	0.124	0.103	0.120	0.151	**S.D.**	0.030	0.0057
%CV	5.0	4.8	4.1	3.4	**%CV**	2.0	14.0

2. **Inter-assay precision:**
   To determine between-run precision, four different samples were assayed by replicates on twelve different assays.

Sample	1	2	3	4	**Control**	RC	NRC
N	12	12	12	12	**N**	12	12
S/CO	2.35	2.21	2.78	4.24	**Mean**	1.457	0.058
S.D.	0.114	0.116	0.273	0.309	**S.D.**	0.051	0.010
%CV	4.9	5.2	9.8	7.3	**%CV**	3.5	18.0

3. **Specificity and Sensitivity:**

The prevalence of anti-HEV IgG in testing sera from well characterised at the early stage of outbreaks of Hepatitis E in China was 95% with this test. Additional evidence of test specificity was obtained by testing samples from persons with HAV, HBV, HCV, and HIV infections. Among thirty-eight such samples, results were negative with these tests.

HEV-IgG antibody can be found in normal healthy individual, which indicates previous exposure to hepatitis E virus.

INTENDED USE AND USER RESPONSIBILITY

This ELISA kit is intended for laboratory research use only. Users should take cautions on result interpretation and should not use the data obtained from this test for patient management and clinic diagnosis.

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