DRG® HEV IgG Antibody ELISA (EIA-1813)

Revised 20 June 2011 rm (Vers. 2.1) **RUO**

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE

The HEV IgG Antibody ELISA (EIA-1813) is to be used for 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 AND/OR THERAPEUTIC PROCEDURES.

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.

1. **MICROTITER PLATE** ***96 wells***
   Pre-coated with HEV antigens

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

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

4. **REACTIVE CONTROL** ***1 mL***

Inactivated human serum.

5. **SAMPLE DILUENT** *12 mL*
Buffered solution with animal serum, preservative and color indicator.

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

7. **SUBSTRATE A** *10 mL*
Buffered solution with H_2O_2.

8. **SUBSTRATE B** *10 mL*
Buffered solution with TMB.

9. **STOP SOLUTION** *14 mL*
2N sulphuric acid (H_2SO_4). Caution: 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.

   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.

2. **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.*

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

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 4.

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

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

11. 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

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