

# HEV IgM ELISA Kit

Enzyme-linked immunosorbent assay for the detection IgM antibody against HEV serum or plasma

## INTENDED USE

Enzyme ImmunoAssay (ELISA) for the determination of IgM antibodies to Hepatitis E Virus in human plasma and sera. The kit may be used for the follow-up of HEV-infected patients.

## SUMMARY AND PRINCIPLE OF THE TEST

Major epidemics of enterically transmitted non-A, non-B hepatitis (ET-NANBH) have been found to occur in developing regions such as Asia, the former USSR, Central America and Africa (1,2). Sporadic cases have also been reported in developed nations, including Australia, the United Kingdom and the United States (3,4,5). Cases in developed nations have generally been associated with travel to endemic regions.

The course of the infection is generally acute and self-limiting without chronic sequelae. There is, however, a high incidence of mortality in pregnant women in the third trimester, about 10-20% (1) and a mortality rate of 1-2% in the general population, which is 10 times that of hepatitis A (HAV). With the cloning of the etiological agent of ET-NANBH and the identification of type common viral epitopes (6,7), specific diagnostic tools have been developed to detect antibodies to hepatitis E virus (HEV). Studies with Egyptian children from Benha in 1986 revealed that previous exposure to HEV will elicit an IgG response (8) which may be transient and disappear in 6 months but can sometimes last up to 8 years or more as seen in a recent study in Taiwan (9). The IgM response has been shown to be limited to the acute phase of HEV infection. Previously, detection of the acute response in HEV infection has been through observation of viral particles in the stool of infected individuals using IEM or by PCR (10, 11). This method requires expensive equipment and technique-dependent expertise. Furthermore, the shedding of the viral particles is usually in small quantities and may not be of sufficient titer to be detected.

These tests are based on synthetic immunodominant antigens derived from conservative regions of the virus. Tests for IgM are used to determine the nature of the infective agent in patients showing symptoms of hepatitis, in order to rule out the possibility of other most severe viral infections (HBV, HDV, HCV).

The assay is based on the principle of "IgM capture" where IgM class antibodies in the sample are first captured by the solid phase coated with anti hIgM antibody. After washing out all the other components of the sample and in particular IgG antibodies, the specific IgM captured on the solid phase are detected by the addition of a purified preparation of HEV Ag conjugated with peroxidase (HRP). After incubation, microwells are washed to remove unbound conjugate and then the chromogen/substrate is added. In the presence of peroxidase the colourless substrate is hydrolysed to a coloured end-product, whose optical density may be detected and is proportional to the amount of IgM antibodies to HEV present in the sample.

## REAGENTS

### Materials provided with the kits:

1. Microtiter Well: coated with goat anti-human IgM.
2. Negative Control: 0.25ml normal serum.
3. Positive Control: 0.25ml anti-HEV IgM positive serum.
4. Enzyme Conjugate: 12 ml, HEV Ag-HRP.
5. Specimne Diluent: 12ml
6. Wash Buffer Concentrate (20x): 40 ml, The buffer should be diluted 20 times with distilled water before use.
7. Substrate Solution A: 6 ml Urea Peroxide
8. Substrate Solution B: 6 ml TMB solution
9. Stop Solution: 6 ml 2N Sulfuric Acid

### Materials required but not provided:

1. Precision pipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled water.
4. Humidified Box capable of maintaining 37°C
5. Absorbent paper or paper towel.
6. Microtiter plate or strip-well washer
7. Microtiter plate reader.

## SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques. Remove serum or plasma

from the clot or blood cells as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Plasma samples containing EDTA, heparin or oxalate may interfere with test procedures and should be avoided. Specimen with extensive particulate should be clarified by centrifugation prior to use. Covered specimens may be stored for up to 48 hours at 2°-8°C prior to assaying. Specimens held for a longer time can be frozen at -20°C for mix prior to testing. Avoid repeated freeze thaw. At least, two wells of negative and positive controls each should be run in every assay.

## PRECAUTIONS

1. Caution: Some components of this kit contain human serum. 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. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
2. Wear disposable gloves while handling kit reagents and specimens and thoroughly wash hands afterwards.
3. Dispose off all specimens and materials used to perform the test as if they contained infectious agents.
4. Do not mix reagents from kits with different lot numbers.
5. Cross contamination between reagents will invalidate the test results.
6. All reagents and components except the conjugate must be equilibrated at room temperature prior to use.

## STORAGE OF TEST KITS AND INSTRUMENTATION

Unopened test kits should be stored at 2°-8°C upon receipt. Micro titer plate, once opened, should be kept in a sealed bag with desiccants to minimize exposure to damp air. To remove the required number of strips from the micro titer plates, bring the sealed pouches to room temperature first and then open the pouches. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. Opened test kits will remain stable until the expiration date shown in 4°C, provided it is stored as described above. A micro titer plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

## WORKING REAGENT PREPARATION, STORAGE AND STABILITY

No reagent preparation is required except for wash buffer, which is supplied as a 20 X concentrate.

## WORKING WASH BUFFER

Dilute the 20X wash buffer concentrate with deionized or distilled water 1:20. For example, 5 ml of wash buffer concentrate should be diluted to a total volume of 100 mL with deionized or distilled water.

## STABILITY OF OPENED KIT COMPONENTS AND DILUTED REAGENTS

The diluted wash buffer is stable for at least one week when stored at room temperature. Substrate is stable for the expiration date of the kit. The micro titer plates should be opened after they have been kept at room temperature for 20-30 minutes. After removing the required number of strips, the plates should be resealed in the foil pouch bags along with the desiccant and stored at 2°-8°C. Exposure of the plates to humidity drastically reduces the shelf life.

## ASSAY PROCEDURE:

1. Allow all components to reach room temperature before use.
2. Dispense 100ul of Positive Control as well as Negative Control in duplicate into respective wells. Set one blank well as background control.
3. Dispense 100ul specimen diluent to test wells.
4. Add 10ul of serum or plasma samples into respective wells. Mix it gently by swirling the microtiter plate on flat bench for 1 min.
5. Place the microtiter plate into a humidified box and incubate at 37°C for 30 min.
6. Wash each well 5 times by filling each well with diluted 1X wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
7. Add 100 ul of Enzyme Conjugate to each well. Mix it gently by swirling the microtiter plate on flat bench for 1 min. Do not add Enzyme Conjugate to the blank well.
8. Place the microtiter plate into a humidified box and incubate at 37°C for 20 min.

9. Wash each well 5 times by filling each well with diluted 1X wash buffer, then inverting the plate vigorously to get all water out and blocking the rim of wells on absorbent paper for a few seconds.
10. Add one drop (50 ul) of Substrate Solution A (HRP substrate) to each well, then add one drop (50 ul) of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 10 min.
11. Add one drop (50 ul) of Stop Solution to each well to stop the color reaction. Read OD values of all samples at 450 nm.

#### INTERPRETATION OF RESULTS

**EIA Reader at 450 nm (using the OD value of the blank well to correct all the OD reading from all wells):**

Calculation of Cut Off Value(COV):  
Mean of the Negative Controls (NCx)+0.10

Positive: the sample OD is equal or higher than COV

Negative: the sample OD is less than the COV

The negative control should be less than OD 0.1, and the positive should be over OD 0.8, or the result is invalid.

#### REFERENCES

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*In vitro* diagnostic device



Consult instructions for use



Keep dry



Temperature limitation



Use by



Lot code



Catalogue number



Contains sufficient for <n> tests



Manufacturer



Do not use if package damaged

#### Manufacturer:

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April 10, 2008 Revision: 03