

HEV Ab ELISA Kit

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

INTENDED USE

The **4th generation** double antigen sandwich Enzyme ImmunoAssay (ELISA) for the qualitative determination of antibodies to Hepatitis E Virus in human plasma and sera. The kit may be used for the screening of blood units and the follow-up of HEV-infected patients.

SUMMARY AND PRINCIPLE OF THE TEST

Hepatitis E Virus or HEV is a recently discovered agent of enterically transmitted viral hepatitis. HEV is an unenveloped single-strand RNA virus structurally similar to Calicivirus and is found in the stool of infected patients. HEV is a serious problem in many developing countries and its first outbreak was reported in 1955 in New Delhi, India.

Hepatitis E has never been associated with chronic infection; however a high case-fatality rate has been found among pregnant women. The cloning and sequencing of HEV genome have led to the development of serological tests for the detection of anti HEV antibodies.

The HEV Ab EIA is a solid-phase simultaneous immunoassay to detect antibody against HEV. Microwells are coated with HEV multiple epitopes synthetic peptide and recombinant antigen. A serum specimen and the specimen diluent are added to the microwells. After incubation, the unbound antibody are washed away, and then add Horseradish Peroxidase (HRP) conjugated HEV antigen, the complex of antigen-antibody-antigen (HRP-conjugated HEV, anti-HEV antibody and HEV on the wells) will be formed.

The unbound enzyme conjugates will be washed away and then the chromogen substrate solution containing urea peroxide is added to the wells. A blue color is developed in proportion to the amount of anti-HEV antibody in the specimens. The enzyme-substrate reaction is stopped by the addition of sulfuric acid. The absorbance of controls and specimens is determined by using EIA reader with wavelength set at 450 nm.

REAGENTS

Materials provided with the kits:

1. Microtiter Well: coated with HEV Ag.
2. Negative Control: 1ml normal serum.
3. Positive Control: 1ml anti-HEV 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 two drop (100 ul) of Positive Control as well as Negative Control in duplicate into respective wells. Set one blank well as background control.
3. Dispense 100 ul 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)+0.15

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.











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Manufacturer:

Biocare Diagnostics Ltd.
 6F, Building B, 108 Xinghua Road
 Xiangzhou, Zhuhai, China 519000
 Tel: +86-756-8238560
 E-mail: info@ivdbiocare.com
 Website: www.ivdbiocare.com

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	<i>In vitro</i> diagnostic device		Lot code
	Consult instructions for use		Catalogue number
	Keep dry		Contains sufficient for <n> tests
	Temperature limitation		Manufacturer
	Use by		Do not use if package damaged