



HEV Ab EIA kit

(For in vitro diagnostic use only)

Enzyme-linked immunosorbent assay for the detection of antibody to HEV

INTENDED USE

HEV Ab EIA is a qualitative enzyme immunoassay for the detection of antibody to HEV in human serum or plasma.

SUMMARY AND PRINCIPLE OF THE TEST

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 antibodies 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 450nm.

REAGENTS

Materials provided with the kits:

1. 8X12 well microtiter strip: 1 plate, coated with HEV antigen.
2. Negative Control: 1 vial.
3. Positive Control: 1 vial.
4. Sample Diluent: 1 vial
5. Enzyme Conjugate: containing HRP-conjugated-HEV for 96 tests
6. Wash Buffer (20X): containing PBS, Tween. The buffer should be diluted with distilled water 1:20 before use.
7. Substrate Solution A: urea peroxide.
8. Substrate Solution B: TMB.
9. Stop Solution: 2N Sulfuric Acid

Materials required but not provided:

1. Micropipettes: 0.02, 0.05, 0.10, 0.15, 0.20, and 1.0 ml.
2. Disposable pipette tips.
3. Distilled or deionized 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 with 450nm wavelength
8. Timer

PRECAUTION FOR USERS

1. For in-vitro diagnostic use only.
2. Do not use kit beyond expiration date.
3. Do not mix components from kits with different lot number.
4. Avoid microbial contamination of reagents.
5. Do not pipette reagent by mouth and no smoking or eating while performing assays.
6. Wear gloves during the whole process and avoid reagents or

specimen spilling-out.

7. Wipe up the spills using 5% hypochlorite solution.
8. Decontaminate all liquids or solid wastes before depositing.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. Either serum or plasma can be used in this test. Remove serum or plasma from the clot or blood cells as soon as possible to avoid hemolysis. Specimen with extensive particulate should be clarified by centrifugation prior to use. Specimen frozen at -20°C or colder may be used. Avoid repeated freeze thaw.

STORAGE OF TEST KIT

Unopened test kits should be stored at 2-8°C. **DO NOT FREEZE KIT COMPONENTS.** The microtiter plate should be kept in a sealed bag to minimize exposure to damp air. Use up the reagents as soon as possible after the kit is unpacked.

ASSAY PROCEDURE

1. Allow all components to reach room temperature before use.
2. Dispense 100 µl of Positive Control as well as Negative Control in duplicate into respective wells.
3. Dispense 100 µl specimen diluent to test and blank well.
4. Set one blank well as background control and 100 µl 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 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 µl 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 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 50 µl of Substrate Solution A (HRP substrate) to each well, and then add 50 µl of Substrate Solution B (TMB) to each well. Mix gently and incubate at 37°C for 10 min.
11. Add one drop (50 µl) 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 .

LIMITATIONS OF THE ASSAY

1. HEV EIA is limited to the detection of antibody against HEV in serum or plasma.
2. As in other sensitive immunoassay, there is the possibility that non-repeatable reaction may occur due to inadequate washing. So do aspirate the well or get rid of the entire content of wells before adding the washing solution.
3. As with all diagnostic tests, a definitive clinical diagnosis should not be made only on the basis of a single test . A complete evaluation by physician is needed for a final diagnosis.

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