Instruction Manual

IgG/IgM anti-HEV EIA

IgG/IgM anti-HEV antibody determination kit by EIA

- Thoroughly read this instruction manual before use of this kit.
- This kit is for research use only.

I. Kit components

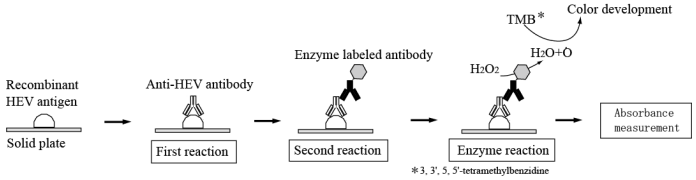
1. Microplate coated with HEV antigen (8 wells/strip x 12)... 1 plate (Recombinant HEV antigen)
2. Negative control (blue) ..... 0.5 mL x 1 vial
3. IgG positive control (green) ..... 0.5 mL x 1 vial
4. IgM positive control (yellow) ..... 0.5 mL x 1 vial
5. Sample diluent ..... 50 mL x 1 vial
6. Anti-IgG enzyme labeled monoclonal antibody ..... 5 mL x 1 vial (Peroxidase labeled anti-human IgG mouse monoclonal antibody)
7. Anti-IgM enzyme labeled monoclonal antibody..... 5 mL x 1 vial (Peroxidase labeled anti-human IgM mouse monoclonal antibody)
8. Enzyme substrate ..... 5 mL x 1 vial (3, 3', 5, 5'-tetramethylbenzidine)
9. Reaction stopper ..... 5 mL x 1 vial
10. 20x concentrated washing solution ..... 50 mL x 1 vial (containing detergent)
11. Plate seal ..... 3 sheets

II. Application

Detection of IgG anti-HEV antibody or IgM anti-HEV antibody in human serum

III. Assay principle

IgG anti-HEV antibody is detected in sera of patients infected by hepatitis E virus (HEV) in the past, and IgM anti-HEV antibody is detected during the acute stage of infection with HEV. The detection system of this kit is based on the enzyme immuno assay (EIA) and is made up of 2 steps of the antigen-antibody reaction and the enzyme coloring reaction. The first antigen-antibody reaction takes place between the HEV antigen coated on the microplate and the anti-HEV antibody in samples and the second reaction between the IgG/IgM anti-HEV antibody bound to the antigen coated on the microplate and the antibody labeled with enzyme (horseradish peroxidase labeled antibody). When the IgG/IgM anti-HEV antibody is present in samples, the first and the second reactions take place and absorbance by color proportional to the amount of the IgG/IgM anti-HEV antibody in samples developed by enzyme reaction is measured.



IV. Sampling precautions

1. Sample collection and storage

1) When collecting blood, avoid hemolysis and separate serum immediately.
2) It is recommended to determine samples on the day collected. Keep frozen if they are stored and avoid frequent freeze-thaw cycles.

2. Interference substances

1) Up to 1,500 degree of chylomicron (formazine turbidity), 15 mg/dL of bilirubin C, 15 mg/dL of bilirubin F, 400 mg/dL of hemolytic hemoglobin, and/or 400 IU/mL of rheumatoid factor do not interfere with the determination.

V. Operation

1. Preparation of reagent

1) Washing solution
Dilute 20x concentrated washing solution 20 times with purified water. Keep this solution at 2 - 10ºC.

2. Materials required but not provided

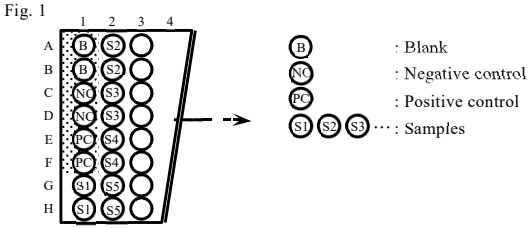
1) Micropipettes, 10 µL, 100 µL, 200 µL, and 1,000 µL
2) A measuring cylinder, 1 L
3) An aspirator and a polyethylene washing bottle, or a microplate washer
4) A dark box (A light tight cupboard or a drawer will do.)
5) A dual-wavelength microplate reader (main wavelength 440 - 460 nm, sub wavelength 620 nm or longer)

3. Assay procedure

Note:

- **Return the kit to 15 - 30ºC before use.**
- **In each assay, prepare blank in 1 well or more, Positive controls in 2 wells or more, and Negative controls in 2 wells or more as illustrated.**

Fig. 1



1) Dilution of samples
Add 5 µL of each samples (serum) to 500 µL of Sample diluent and thoroughly mix using pipettes.

2) Addition of samples
Add 50 µL of each diluted samples, Positive control, or Negative control into the wells.
Use IgG positive control in the case of determination of IgG anti-HEV, and use IgM positive control in the case of determination of IgM anti-HEV.

Note

- **Each of the 12 strips can be detached from their holder. Use only required number of strips and keep at 2 - 10ºC remaining strips in an air tight aluminum pouch with desiccant.**

3) 1st reaction
Cover the microplate wells with the plate seal provided and leave them to stand at 15 - 30ºC for 1 hour.

4) Washing
Remove the plate seal and suck out the well contents using an aspirator. Fill each well with the washing solution prepared in **1. Preparation of reagent** using a washing bottle and shake out the solution by holding the microplate wells upside down. Repeat this washing 5 times. When the microplate is washed using a microplate washer, wash 5 times.

Note

- **While washing the microplate wells, care should be taken not to dry inside the wells. After washing, follow the next step below.**

5) Addition of the enzyme labeled monoclonal antibody
Add 50 µL of the enzyme labeled monoclonal antibody in all wells but not blank well.
Use Anti-IgG enzyme labeled monoclonal antibody in the case of determination of IgG anti-HEV, and use Anti-IgM enzyme labeled monoclonal antibody in the case of determination of IgM anti-HEV.

6) 2nd reaction
Cover the microplate wells with the plate seal provided and leave it to stand at 15 - 30ºC for 1 hour.

7) Washing
Wash the microplate wells as in **4)** above.

8) Addition of Enzyme substrate
Add 50 µL of Enzyme substrate in all wells.

9) Enzyme reaction
Cover the microplate with the plate seal provided. Leave the microplate to stand at 15 - 30ºC in the dark for 30 min.

10) Addition of Reaction stopper
Remove the plate seal and add 50 µL of Reaction stopper in all wells and thoroughly mix.

11) Absorbance measurement
Measure absorbance of each well (main wavelength 450 nm /or 440 - 460 nm, sub wavelength 630 nm).

Note

- **Measure absorbance within 30 min after stopping the reaction.**

Assay Procedure and Well Arrangement

Well Arrangement		Blank	Negative control・Positive control	Samples
	Wells	1A, (1B)	1C - 1F	1G - 12H
1	Dilution of samples	–	No dilution needed	101 times dilution
2	Addition of sample or controls Negative control Positive control Diluted sample	 – – –	 50 µL 50 µL –	 – – 50 µL
3	1st reaction	1 hr at 15 - 30ºC		
4	Washing	5 times		
5	Addition of the enzyme labeled monoclonal antibody	–	50 µL	50 µL
6	2nd reaction	1 hr at 15 - 30ºC		
7	Washing	5 times		
8	Addition of Enzyme substrate	50 µL		
9	Enzyme reaction	30 min in the dark at 15 - 30ºC		
10	Addition of Reaction stopper	50 µL		
11	Absorbance measurement	Main wavelength 450 nm, sub wavelength 630 nm		
12	Interpretation of results			

VI. Interpretation of results

1. Calculation of cut off value (COV)

1) Calculate the Net OD of positive controls and negative controls, and each sample in accordance with ([absorbance of control or each sample - the mean absorbance of the blank]).
2) Determine COV from the Net OD.

IgG COV=[the mean Net OD of IgG positive controls – the mean Net OD of negative controls] x 0.13
IgM COV=[the mean Net OD of IgM positive controls – the mean Net OD of negative controls] x 0.30

2. Interpretation of results

1) Interpret regarding the following criteria of Net OD of sample for each assay.

- A cut off index (COI) also can be used, according to cut off value (COV) for interpretation.
  COI = (Net OD of each sample) / COV

Criteria of Net OD of sample	COI	Interpretation
COV or more	1 or more	Positive
less than COV	less than 1	Negative

3. Precaution in interpretation

1) If samples are in window period or immunity has decreased, the interpretation may be negative, even though it includes IgG/IgM anti-HEV antibody.
2) It may be occurred non-specific reaction in case of sample from autoimmune disease patient.

VII. Warnings and precautions

This kit must be used according to the instructions and for the purpose described in this manual. No result is guaranteed in any use or for any purpose other than those described in this manual.

1. General precautions

1) Make sure to return the kit to the 15 - 30ºC before use.
2) Do not mix up kit components of different production lots. Do not reuse microplate wells.
3) Assay strictly as instructed.
4) Do not use expired reagents.
5) Avoid contamination of the kit reagents with microorganisms.
6) Thoroughly wash equipments used for the assay and rinse them with purified water.
7) Replace micropipette tips for each sample and reagent.

2. Operational precautions

1) Measure absorbance of blank, positive control, and negative control in each assay.
2) Once assay is started, complete it within the prescribed time. Care should be taken to allow the same reaction time for all samples.
3) Make sure that all reactions take place at 15 - 30ºC.
4) Measure absorbance within 30 min after stopping the enzyme reaction.
5) Do not scrape the microplate or touch the bottom of wells. Do not dry the surface of the wells during operation.

3. Handling precautions

1) Although Positive controls and Negative control in this kit are tested negative for HBV, HCV antibody, HIV-1 antibody, and HIV-2 antibody, handle them carefully as if they were infectious with these viruses.
2) Samples should be handled as if they were potentially infectious with HBV, HCV, and HIV.
3) Avoid contact of reagents. If they contact skin, wash with plenty of water. (They are toxic and irritable and burn skin or mucous membrane.) Get medical care if need.
4) Samples, reagents, and materials used for the assay should be treated with either of the followings.
   a) Immerse in 0.05% formalin solution at 37ºC for over 72 hrs.
   b) Immerse in 2 w/v% glutaraldehyde solution for over 1 hr.
   c) Immerse in sodium hypochlorite solution (concentration of effective chlorine: 1,000 ppm or more) for over 1 hr.
   d) Autoclave at 121ºC for 20 min.
5) Sample diluent, Negative control, and Positive controls contain sodium azide. Flush drains with a sufficient volume of water to prevent forming of explosive metal azide after disposing of them.
6) Dispose of container and unused contents in accordance with federal, state and local requirements.
7) If sample or used reagent are spilled, wipe by using sodium hypochlorite solution (concentration of effective chlorine: 1,000 ppm or more) or glutaraldehyde (immerse in 2 w/v%, for over 1 hr), and sterilize.

VIII. Storage and shelf life

Store the kit at 2 - 10ºC and avoid freezing. This kit is stable for 1 year after the date of manufacture. Validity of kit is shown in the package.

IX. Package

1 kit for 96 tests Code No. 1Z23

X. Reference

1) Mikhail S, Balayan MD: Type E Hepatitis: State of the Art. Int J Infect Dis **2** (2): 113-120, 1997.
2) Mizuo H, Suzuki K, Takikawa Y, et al: Polyphyletic strains of hepatitis E virus are responsible for sporadic cases of acute hepatitis in Japan. J Clin Microbiol **40**: 3209-3218, 2002.
3) Takahashi M, Nishizawa T, Miyajima H, et al : Swine hepatitis E virus strains in Japan form four phylogenetic clusters comparable with those of Japanese isolates of human hepatitis E virus. J Gen Virol **84** : 851-862, 2003.
4) Takahashi M, Kusakai S, Mizuo H, et al : Simultaneous detection of immunoglobulin A (IgA) and IgM antibodies against hepatitis E virus (HEV) is highly specific for diagnosis of acute HEV infection. J Clin Microbiol **43** : 49-56, 2005.

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