Instruction Manual § Research use only §

IMMUNIS® HGF EIA

Reagent for determination of human Hepatocyte Growth Factor (HGF)

- This kit is intended for research use only, not for diagnostic use.
- Read this instruction manual thoroughly before use. 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.
- Use any equipment for the assay according to its instruction manual.

I. General description

Serum Hepatocyte growth factor level elevates in various liver diseases and fluctuates reflecting the conditions of patients. Serum HGF concentration in fulminant hepatitis patients increases significantly in comparison with those in other hepatitis and its close relation with encephalopathy is known widely. That is, this marker shows an abnormal elevation just before expression of encephalopathy and decreases with recovery from it. After expression of encephalopathy, HGF level further elevates when poor prognosis is expected. Since prothrombin time as one of the diagnostic criteria of fulminant hepatitis also decreases in acute hepatitis, liver cirrhosis, or hepatocellular carcinoma, HGF is expected to be a marker more specific to fulminant hepatitis.

Determination of HGF, therefore, is an effective means for diagnosis of fulminant hepatitis at its early stage and its prognosis.

"IMMUNIS® HGF EIA" affords determination of serum HGF at ease by means of the enzyme immunoassay (EIA) using monoclonal antibodies.

Features:

1. Two monoclonal antibodies used in this kit assure determination of HGF level at high sensitivity.
2. 2-step immuno reaction assures determination in a short time.
3. This kit does not require any special facility and can determine the marker at least with the minimum preparation of reagents.

II. Kit components

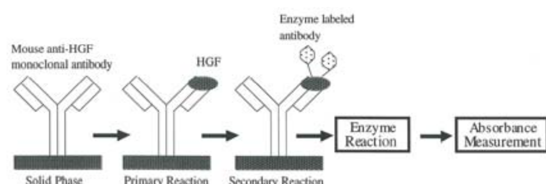
1. Anti-HGF monoclonal antibody coated microplate (8 wells x 12 strips)
   (Anti-HGF mouse monoclonal antibody) .... 1 plate
2. HGF standard solution 1 (0 ng/mL) .... 0.5 mL x 1 vial
   HGF standard solution 2 (0.1 ng/mL) .... 0.5 mL x 1 vial
   HGF standard solution 3 (0.3 ng/mL) .... 0.5 mL x 1 vial
   HGF standard solution 4 (1 ng/mL) .... 0.5 mL x 1 vial
   HGF standard solution 5 (3 ng/mL) .... 0.5 mL x 1 vial
3. Sample diluent .... 15 mL x 1 vial
4. Enzyme labeled monoclonal antibody .... 10 mL x 1 vial
   (Peroxidase labeled anti-HGF mouse monoclonal antibody)
5. Enzyme substrate .... 30 mL x 1 vial
   (Sodium perborate tetrahydrate)
6. Color developer .... 4 tablets
   (o-phenylendiamine dihydrochloride)
7. Reaction stopper .... 6 mL x 1 vial
   (2 mol/L sulfuric acid)
8. 20x concentrated washing solution .... 25 mL x 2 vials
9. Plate seal .... 3 sheets

III. Application

Determination of HGF concentration in human serum

IV. Assay principle

This kit adopts two-step sandwich EIA using anti-HGF mouse monoclonal antibodies by immunization with recombinant HGF. In the first antigen-antibody reaction, anti-HGF mouse monoclonal antibody immobilized on the microplate reacts with HGF in samples and in the second antigen-antibody reaction, HGF adsorbed on the monoclonal antibody on the microplate reacts with the enzyme labeled monoclonal antibody (peroxidase labeled anti-HGF mouse monoclonal antibody). In the enzyme reaction to follow, coloring substance is formed in proportion to the amount of HGF in samples. After a period of time, the coloring reaction is stopped to measure absorbance of the substance. HGF concentration in samples is determined by a calibration curve prepared using the HGF standard solution.



V. Operational precautions

1. Sample collection and storage

1) When collecting blood, avoid hemolysis and separate serum immediately. It is recommended to use fresh sera for the assay.
2) For collection and storage of samples, use polypropylene tubes, polyethylene tubes, or silicon coated glass tubes.
3) Samples can be stored at 4 ºC for one week. If they need to be stored for more than one week, keep them at -20 ºC or below. Avoid frequent freeze-thaw cycles.
4) Return samples to the room temperature if they are stored in cool/frozen condition.

2. Interference substances

1) Up to 1,500 FTU of chyle, 4.0 mg/dL of bilirubin C, 20.0 mg/dL of bilirubin F, or 500 mg/dL of homolytic hemoglobin did not interfere with the determination.

3. Operational precautions

1) Check accuracy of equipment for measurement and properly use them according to its instruction.
2) Thoroughly wash equipment used for the assay and rinse them with purified water.
3) Some white powder which might be found in the well of the microplate does not affect the result.
4) Replace micropipette tips for each sample and reagent.
5) Assay with HGF standard solutions in each assay.
6) Thoroughly mix with pipetting after adding samples.
7) Once assay is started, complete it within the prescribed time, allowing the same reaction time for all samples.
8) Make sure that all reactions take place at 20 ~ 30 ºC.
9) Do not use a melted or brown-colored Color developer.
10) Do not make react with any other enzyme immuno assay system using color developers contain organic sorbent, such as Tetramethylbenzidine. The reaction of OPD may be inhibited and it may affect the results.
11) Measure absorbance within 2 hrs after stopping the color development.
12) Do not scrape the microplate or touch the bottom of wells, nor dry up the inner surface of the wells during operation.

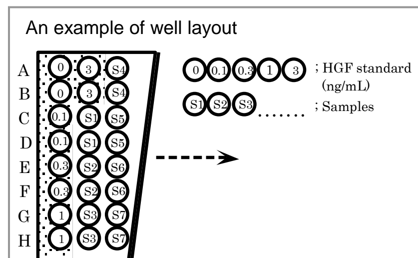
VI. Operation

1. Preparation of reagents

1) Enzyme substrate solution (containing the color developer)
   Add one tablet of the color developer into 3 mL of the enzyme substrate and leave the solution in a dark place. Thoroughly mix the solution after all babbles are gone. Prepare a sufficient volume of this solution to cover all wells to be used for the test.
   This solution must be prepared 15 min before use and used up within 60 min.
2) Washing solution
   Dilute 20x concentrated washing solution 20 times with purified water. Keep this solution at 2 ~ 10ºC.
3) Return reagents of the kit to 20 ~ 30ºC before use.
4) The microplate can be divided into 12 strips. Strips not used should be kept in an aluminum pouch together with desiccant and stored at 2 ~ 10ºC.
5) Color developer not used should be kept in an aluminum pouch together with desiccant and stored at 2 ~ 10ºC.

2. Materials required but not provided

1) Micropipettes, 50 µL and 100 µL
2) A measuring pipette, 10 mL
3) A measuring cylinder, 1 L
4) A microplate shaker
5) An aspirator and a polyethylene washing bottle, or a microplate washer
6) A dark box (A light tight cupboard or a drawer will do.)
7) A microplate reader (main wavelength 492 nm or 490 nm, sub wavelength 620 nm or longer)
8) Logarithmic scale



3. Assay procedure

Note: For each test, determine absorbance of the standard solution (2 wells x 5 concentrations = 10 wells) as illustrated below.

1) Addition of Sample diluent
   Pipette 50 µL each of Sample diluent in the wells for the HGF standard solution and wells for samples.
2) Addition of HGF standard solutions and samples
   According to the well layout, dispense 50 µL each of HGF standard solutions and samples to each wells respectively.

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Dilution of samples with high HGF concentration
Samples that are expected to overshoot the range of the calibration curve should be diluted in advance with Sample diluent.

3) Primary reaction
Cover the microplate with the plate seal provided and shake the microplate on a microplate shaker at 200 ~ 700 rpm for 1 hr at 20 ~ 30°C.

4) Washing
Remove the plate seal and suck out the well contents with an aspirator. Wash all wells 5 times with the washing solution prepared under **"2) Washing solution"** in **"1. Preparation of reagents"**, using polyethylene washing bottle, or a microplate washer. Then tap the microplate upside down on a clean paper towel.
Follow the next step immediately so as to avoid drying up the wells.

5) Addition of Enzyme labeled monoclonal antibody
Pipette 100 µL of Enzyme labeled monoclonal antibody to each well.

6) Secondary reaction
Cover the microplate with new plate seal and shake the microplate on a microplate shaker for 1 hr at 20 ~ 30°C.

7) Preparation of the enzyme substrate solution (containing the color developer)
Approximately 15 min before the end of the secondary reaction, prepare the enzyme substrate solution (containing the color developer) according to the instruction given **"1) Enzyme substrate solution (containing the color developer)"** in **"1. Preparation of reagents"**.

8) Washing
Repeat the procedure 4) above to wash the microplate wells.

9) Addition of the enzyme substrate solution
Pipette 100 µL of the enzyme substrate solution (containing the color developer) prepared under 7) above to each well.

10) Enzyme reaction
Cover the microplate with new plate seal and leave the microplate at 20 ~ 30°C in the dark for 30 min.

11) Addition of Reaction stopper
Remove the plate seal and stop color development by adding 50 µL of Reaction stopper in all wells, and mix sufficiently.

12) Absorbance measurement
Measure the absorbance of each well at 492 nm or 490 nm. When a dual wavelength microplate reader is used, set the reference wavelength at 620 nm or longer.

Scheme of assay procedure

		HGF Standard Solution	Samples
1	Assignment of Wells	1 A ~ 1 H, 2 A, 2 B	2 C ~ 12 H
2	Addition of HGF standard solution and samples Sample diluent HGF standard solution Samples*	 50 µL 50 µL -	 50 µL - 50 µL
3	Primary reaction	for 1 hr at 20 ~ 30°C, shaking	
4	Washing	5 times	
5	Addition of Enzyme labeled monoclonal antibody	100 µL	
6	Secondary reaction	for 1 hr at 20 ~ 30°C, shaking	
7	Washing	5 times	
8	Addition of the enzyme substrate solution (containing the color developer)	100 µL	
9	Enzyme reaction	for 30 min at 20 ~ 30°C in the dark	
10	Addition of Reaction stopper	50 µL	
11	Absorbance measurement	492 nm or 490 nm	
12	Determination		

*Include samples diluted in advance.

VII. Determination

1. Validation of the assay and calculation of the specific absorbance (Net OD)

1) Make sure that absorbance of the Standard solution 1 (0 ng/mL) is less than 0.050.
2) Calculate the mean value of the 2 absorbance readings of the Standard solution 1. If either of the reading is 0.050 or more, disregard that reading and use the other reading. When both readings are 0.050 or more, the assay is void and should be repeated again.

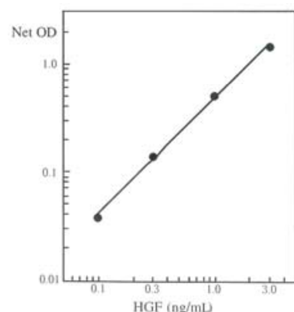

Fig. Example of calibration curve

3) Subtract the mean absorbance reading (or one reading) of the Standard solution 1 from the reading of each well to calculate the Net OD.

2. Calibration curve
Using logarithmic scales, prepare a calibration curve by plotting concentration of the standard solution on the X axis and Net OD on the Y axis.

3. Calculation of HGF concentration
1) Apply the Net OD of each sample to the calibration curve and read HGF concentration.
2) When absorbance of a sample exceeds that of the HGF standard solution 5 (3 ng/mL), dilute the sample and determine it again.

VIII. Specifications

1. Sensitivity
When the HGF standard solution 1 (0 ng/mL) and the HGF standard solution 2 (0.1 ng/mL) are measured 3 times each at 492 nm, there exists the following relation:
(mean value - SD* x 2) of the HGF standard solution 2 (0.1 ng/mL) > (mean value + SD* x 2) of the HGF standard solution 1 (0 ng/mL)
*SD: standard deviation

2. Specificity
When this kits determines 3 panel sera (L, M and H), this kit shows a result within ±25% of the known concentration for the serum L, and within ±20% of that for the sera M and H.

3. Simultaneous reproducibility
When this kits determines panel sera 10 times, CV value of HGF concentration determined is 15% or below.

4. Determination range
Determination range is between 0.1 ng/mL and 3 ng/mL of the calibration curve. Samples of higher HGF concentration than this must be diluted in advance with Sample diluent.

IX. Warnings and precautions

1. General precautions
1) Keep the kit in the condition as instructed and avoid freezing. Keep the reagents in the kit in the dark.
2) HGF standard solutions should be kept with caps at 2 ~ 10°C.
3) Do not use expired reagents.
4) Do not mix up kit components of different production lots.
5) Avoid contamination of the kit reagents with microorganisms.

2. Handling precautions
1) Sera are potentially infectious with HBV, HCV, and HIV. Take care not to touch samples with bare hands or let them splash into the eye. Use disposable gloves in handling samples, and wash your hands thoroughly after completing the test.
2) Avoid contact of reagents. If they contact skin, wash with plenty of water. Get medical care if necessary.
3) Test sera, reagents and the materials used for the assay should be treated with either of the followings.
   a) Immerse in 0.05 w/v% formalin solution at 37°C 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 121°C for 20 min.
4) When discarding Sample diluent and HGF standards, run sufficient volume of tap water, as they contain 0.1% sodium azide.
5) Dispose of container and unused contents in accordance with federal, state and local requirements.

X. Storage and shelf life
Store the kit at 2 ~ 10°C in the dark and avoid freezing.
This kit is stable for 1 year after the date of manufacture. Validity of kit is shown in the package.

XI. Package
1 kit for 96 tests Code No. 1EH1

XII. Reference

1) Nakamura T, Nawa K, Ichihara A : Partial purification and characterization of hepatocyte growth factor from serum of hepatectomized rats. Biochem. Biophys. Res. Commun. **122** : 1450-1459, 1984.
2) Nakamura T, Nishizawa T, Hagiya M, et al : Molecular cloning and expression of human hepatocyte growth factor. Nature **342** : 440-443, 1989.
3) Nakamura T : Structure and function of hepatocyte growth factor. Prog. Growth Factor Res. **3** : 67-85, 1991.

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