# IMMUNIS® HGF EIA

Reagent for determination of Hepatocyte Growth Factor (HGF)

**Thoroughly read this instruction manual before use of this kit.**

I Background of the development and features.

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.

This kit affords determination of serum HGF at ease by means of the enzyme immuno assay 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 configuration.

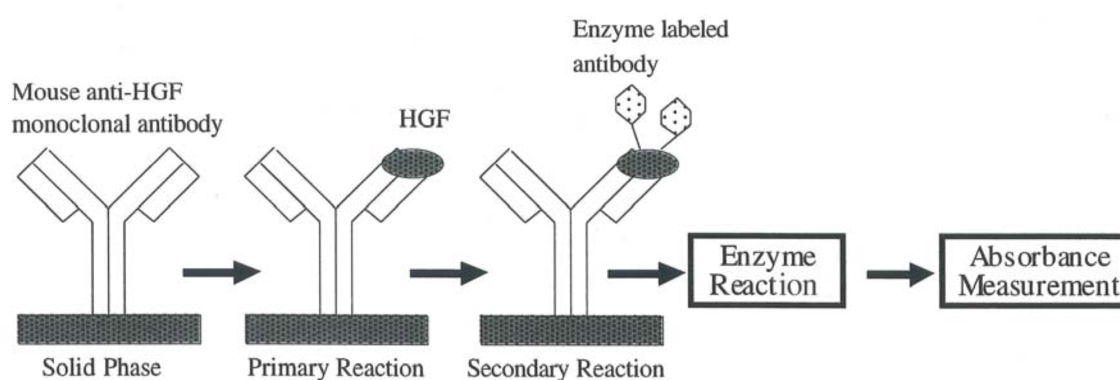
1. Anti-HGF monoclonal antibody coated microplate (8 wells/strip x 12) ............ 1 ea.
   Anti-HGF mouse monoclonal antibody
2. HGF standard solution 1 (0 ng/mL) ................................................ 0.5 mL × 1 vial
   HGF standard solution 2 (0.1 ng/mL) .............................................. 0.5 mL × 1 vial
   HGF standard solution 3 (0.3 ng/mL) .............................................. 0.5 mL × 1 vial
   HGF standard solution 4 (1.0 ng/mL) .............................................. 0.5 mL × 1 vial
   HGF standard solution 5 (3.0 ng/mL) .............................................. 0.5 mL × 1 vial
3. Sample diluent ........................................................................ 15 mL × 1 vial
   Fetal calf serum
4. Enzyme labeled monoclonal antibody................................................ 10 mL× 1 vial
   Peroxidase labeled anti-HGF mouse monoclonal antibody
5. Enzyme substrate .................................................................... 30 mL × 1 vial
   Sodium perborate tetrahydrate
6. Color developer ........................................................................4 tablets
   O-phenylendiamine dihydrochloride
7. Reaction stopper ........................................................................ 6 mL× 1 vial
   2 mol/L sulfuric acid
8. Washing solution (20 times concentrated).........................................25 mL × 2 vials
   Sodium chloride
9. Plate seal ............................................................................................ 3 ea.

III Effect.

Determination of serum HGF concentration.

IV Principles of determination.

Principles of determination by this kit is of Enzyme Immuno Assay (EIA) using mouse monoclonal antibodies expressed by immunization of mouse with HGF produced by gene recombination and consists of two-step antigen-antibody reaction and enzyme coloring reaction. In the first antigen-antibody reaction, mouse anti-HGF 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 enzyme labeled monoclonal antibody (peroxidase labeled mouse anti-HGF monoclonal antibody). In the enzyme reaction to follow, coloring substance is formed in proportion to the amount of HGF in samples. After a given 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.



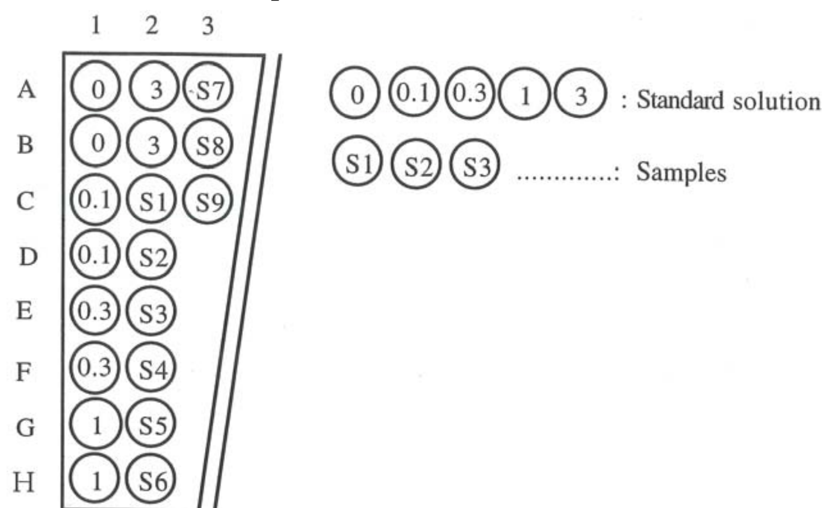

V Preparation of reagents and operation.

1. Tools and equipment required (but not supplied with the kit).
   - Micropipettes, 50 μL and 100 μL.
   - A measuring pipette, 10 mL.
   - A measuring cylinder, 1 L.
   - An aspirator and a polypropylene washing bottle, or a microplate washer.
   - A microplate mixer.
   - A dark box (a light tight cupboard or drawer will do).
   - A microplate reader (capable of reading absorbance at 492 nm or 490 nm).
2. 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 the concentrated washing solution 20 times with purified water. Store at 2 ~ 10°C after preparation.

3. Determination.
   Make sure to return the kit to the room temperature before use. For each test, determine absorbance of the standard solution (2 wells × 5 concentrations = 10 wells) as illustrated below.

   1) Addition of the sample diluent.




   Dispense 50 μL each of the sample diluent in the wells for the HGF standard solution and wells for samples to be tested.

   2) Addition of the HGF standard solution and samples.
   Dispense 50 μL each of the HGF standard solution and samples in respective wells.

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

   Note
   The microplate is supplied with detachable 12 strips of 8 wells each. Any unused strips should be stored in an aluminum pouch with desiccant and kept at 2 ~ 10$^{o}$C. Vials of the HGF standard solution should be sealed with a cap and kept at 2 ~ 10$^{o}$C after use.

   3) Primary reaction.
   Cover the microplate with the plate seal(s), set it securely on the microplate mixer and shake it at 200 ~ 700 rpm for 1 hr at 20 ~ 30$^{o}$C.

   4) Washing.
   Remove the plate seal(s) and suck out the well contents with an aspirator. Fill wells with the washing solution prepared under 2) of V 2. Preparation of reagents above. Hold the microplate up side down and shake out the washing solution. Repeat this 5 times to wash the wells. Wash the wells also 5 times when a microplate washer is used. Finally, tap the microplate surface on a dry, clean paper towel to completely remove the washing solution from the wells.

   Note
   While washing the microplate wells, make sure not to dry inside the wells. Immediately follow the next step as soon as washing is completed.

   5) Addition of the enzyme labeled monoclonal antibody.
   Dispense in the all wells 100 μL each of the enzyme labeled monoclonal antibody.

   6) Secondary reaction.
   Cover the microplate with the plate seal(s), set it on a microplate mixer and shake it for 1 hr at 20 ~ 30$^{o}$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 under 1) of V 2. Preparation of reagents above.

8) Washing.
Wash the microscope wells in the manner given under 4) above.

9) Addition of the enzyme substrate solution (containing the color developer).
Dispense in the all wells 100 µL each of the enzyme substrate solution (containing the color developer) prepared under 7) above.

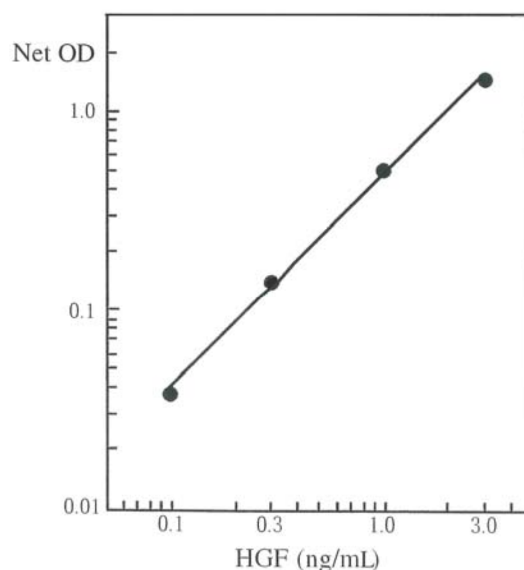
10) Enzyme reaction.
Cover the microplate with the plate seal(s) and leave it to stand at 20 ~ 30°C for 30 min in the dark.

11) Addition of the reaction stopper.
Dispense 50 µL of the reaction stopper in the all wells and thoroughly mix.

12) Absorbance measurement.
Measure by a microplate reader absorbance of each well (at wavelength 492 nm or 490 nm). Absorbance should be measured within 2 hr after addition of the reaction stopper.

VI Results and 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.
   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. 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 (3.0 ng/mL), dilute the sample and determine it again.
4. Summary of assay procedures.

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

*Include samples diluted in advance.

VII Assay precautions.

1. Sample collection.
   1) Collect samples avoiding hemolysis and isolate serum without loss of time.
   2) For collection and storage of samples, use polypropylene tubes or silicon coated glass tubes.
2. Sample handling.
   Samples can be stored at 4$^o$C for one week. If they have to be stored for more than one week, keep them at -20$^o$C or less. Avoid repeated freezing and thawing of samples.
3. Interference substances.
   Up to 1,500 FTU of chylomicron, 4.0 mg/dL of bilirubin C, 20.0 mg/ dL of bilirubin F and 500 mg/ dL of hemoglobin do not adversely affect the determination.
4. Use sera as samples for this kit.

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 - standard deviation x 2) of the HGF standard solution 2 (0.1 ng/mL) > (mean value + standard deviation x 2) of the HGF standard solution 1 (0 ng/mL).
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 0.1 ng/mL in the lowest limit and 3.0 ng/mL in the highest limit of the calibration curve. Samples of higher HGF concentration than this must be diluted in advance with the sample diluent.

IX Handling cautions.

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

1. Cautions in assays.
   1) Check accuracy of tools and properly use them according to their instructions.
   2) All samples should be handled as if they were capable of transmitting HBV, HCV and/or HIV and all tools must be immersed in and sterilized with 0.1% sodium hypochlorite.
   3) Prevent contact of the enzyme substrate, color developer and reaction stopper with skin and mucous membrane. They are toxic and irritant and may cause burn.
2. Cautions in the use of the kit.
   1) Do not use expired reagents.
   2) Do not use reagents of different production lots.
3. Cautions after the use of the kit.
   1) The reaction stopper is highly acidic and should be handled and disposed of with extreme care.
   2) The sample diluent and HGF standard solution contain sodium azide. When discarding them, flush them with a sufficient amount of water to prevent formation of explosive metal azide.
4. Others.
   1) Vials and tools contained in this kit should not be used for other purposes.
   2) Overall diagnosis should be made not only by the results of this assay but by taking into consideration clinical findings and the results of other tests.

X Storage and shelf life.

Store at 2 ~ 10 °C.

This kit is stable for 12 months after the date of manufacture and must be used up before the expiry date shown on the package.

XI Package.

1 kit for 96 tests Code No. 1EH1

XII References.

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

Institute of Immunology Co., Ltd.
1-1-10, Koraku, Bunkyo-Ku,
Tokyo 112-0004, Japan
Tel: +81-3-3814-4081
Fax: +81-3-3814-5957