Instruction Manual for MOUSE HGF EIA

Mouse HGF Determination EIA Kit for Research Purpose

General Description

Hepatocyte growth factor (HGF), which is a potent growth factor as a trigger for liver regeneration, act as accelerator for growth of various cells not only hepatocyte but also epithelial cells, endothelial cells, and some of mesenchymal tissue. Several studies have reported that HGF had correlation with tumor progression. It also established that HGF enhanced the motility of various types of cells, induced morphogenesis and tubule formation, and controlled the immune response, and many researchers have studied using mice or rats.

This kit is developed for determination of mouse hepatocyte growth factor (mouse HGF) for research purposes. Mouse HGF can be determined quantitatively using the mouse HGF standard solution supplied with this kit.

This kit also cross reacts with human HGF but its cross reactivity is about 4%.

Kit Composition

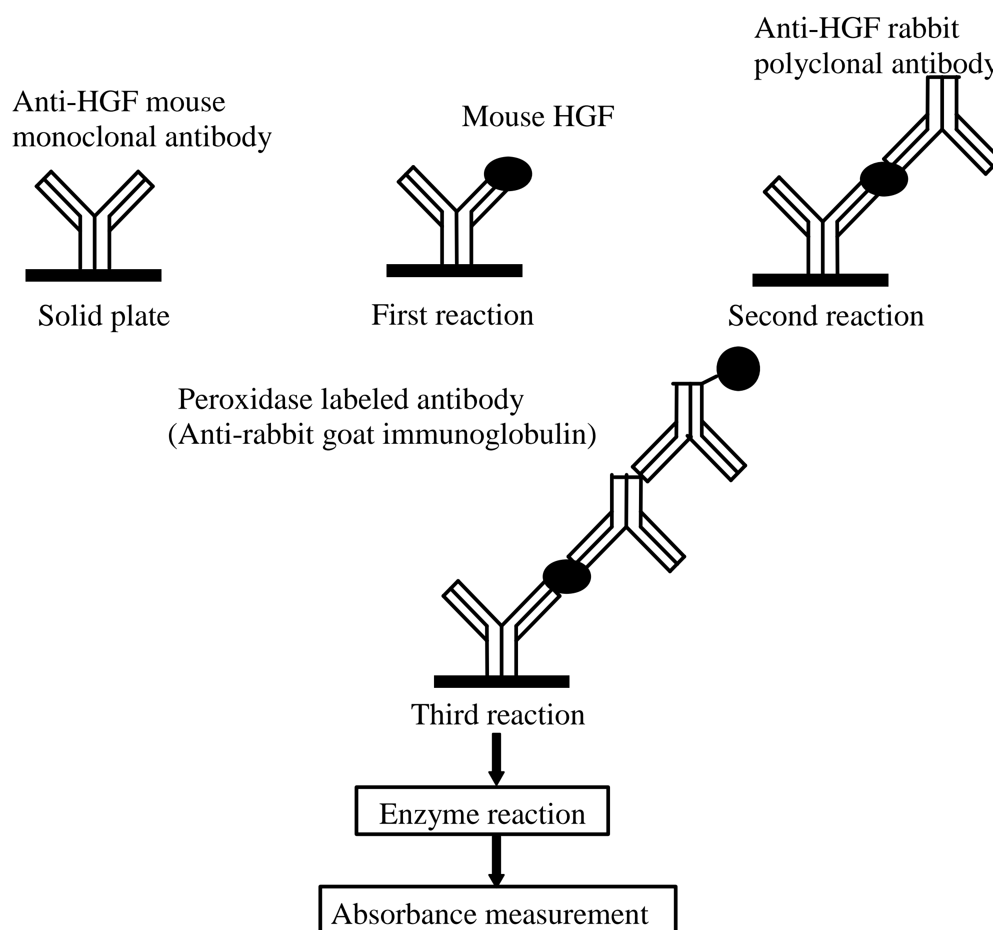
1. **Solid phase microplate (8 wells/strip × 12)** ....1 ea.
   (Coated with anti-HGF monoclonal antibody)
2. **Mouse HGF standard solution**...6 vials
   (1 vial each of 0, 0.4, 1.0, 3.0, 10 and 25 ng/mL standard
   concentration: 0.5 mL/vial)
3. **Sample diluent L, low salt concentration**..15 mL x 1 vial
4. **Sample diluent H, high salt concentration**...15 mL x 1 vial
5. **Anti-HGF rabbit antibody** ....10 mL x 1 vial
   (Anti-HGF rabbit polyclonal antibody)
6. **Enzyme labeled antibody**...10 mL x 1 vial
   (Peroxidase labeled anti-rabbit goat immunoglobulin)
7. **Enzyme substrate solution**..30 mL x 1 vial
   (Sodium perborate, tetrahydrate)
8. **Color developer** ....4 tablets
   (o-phenylenediamine, dihydrochloride)
9. **Reaction stopper solution** ....5 mL x 1 vial
   (19.6% sulfuric acid)
10. **Washing solution** (20 times concentrated) ....25 mL x 3 vials
11. **Plate seal**...3 ea.

Application

For determination of mouse hepatocyte growth factor (mouse HGF).

Assay Principle

The detection system of this kit is of a sandwich configuration based on enzyme immuno assay consisting of three steps of antigen-antibody reaction between a monoclonal antibody raised against genetically engineered human HGF and most cross-reactive with mouse HGF (anti-HGF mouse monoclonal antibody), mouse HGF in samples, anti-HGF rabbit antibody and anti-rabbit goat immunoglobulin, followed by color development by enzyme reaction. The 1st reaction takes place between the anti-HGF mouse monoclonal antibody immobilized on a solid phase microplate and mouse HGF in samples; the 2nd reaction between mouse HGF in samples bound to the solid plate antibody and anti-HGF rabbit polyclonal antibody, and the third reaction between the enzyme labeled antibody (peroxidase labeled anti-rabbit goat immunoglobulin) and the anti-HGF rabbit polyclonal antibody. After the third reaction, color proportional to the mouse HGF concentration is developed by enzyme reaction and its absorbance is measured for the determination of mouse HGF on a calibration curve prepared using the mouse HGF standard solution.



Preparation of reagents and operation

1. **Materials required for assay but not supplied with this kit**

    1) One each of micro pipettes, 50 μL and 100 μL
    2) A measuring pipette, 10 mL
    3) A measuring cylinder, 1 L
    4) An aspirator and a polyethylene washing bottle, or a microplate washer
    5) A dark box (A light tight cupboard or a drawer will do.)
    6) A microplate reader capable of reading wavelength at 492 nm or 490 nm (reference wavelength at 620 nm or longer)

2. **Preparation of reagents**

1) Enzyme substrate solution (containing color developer)

Dissolve one tablet of Color developer per 3 mL of Enzyme substrate solution and leave it to stand in the dark. After bubbles are gone, thoroughly mix the solution. Prepare a sufficient volume (100 μL/well) of this solution to fill all wells to be used. This solution should be prepared 15 min before use and used up within 60 min after preparation.

2) Washing solution

Dilute Washing solution 20 times with purified water. Keep this solution at 2 - 10°C after use.

3. **Operation**

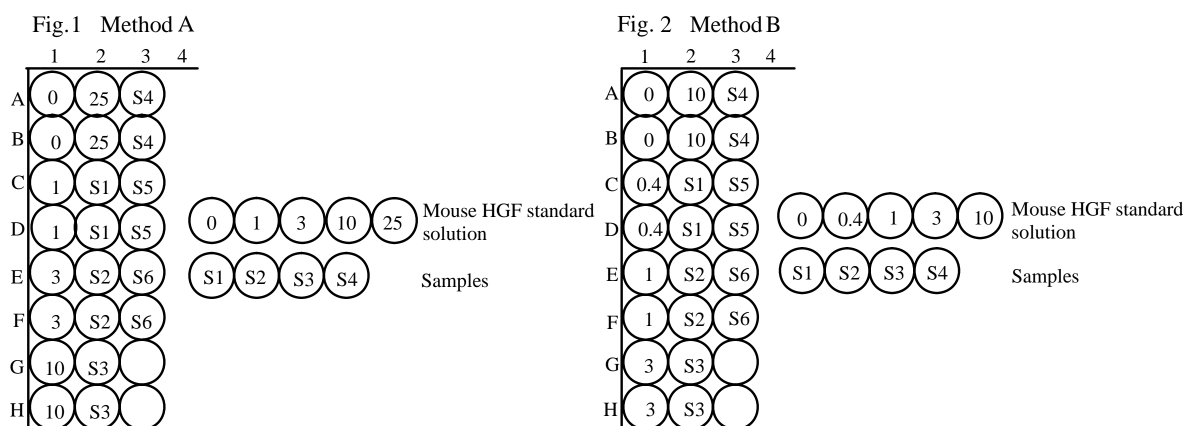
For the determination of samples*1 in high salt concentration buffer, for example, extraction from organs, follow the instruction for high salt concentration samples (***Method A***), and for samples in low salt concentration buffer, for example, plasma*2, follow the instruction for low salt concentration samples (***Method B***). Refer to Appendix 1 for extraction HGF in the tissue sample, or and Appendix 2 for purification of it.
Samples which may show absorbance beyond the calibration curve should be diluted with the sample diluent before assay.

*1: Keep samples in plastic vials such as polyethylene and polypropylene. Do not use glass vials.

*2: Platelet is rich with mouse HGF and can be easily damaged during isolation of plasma. To prevent mixture with platelet derived HGF, isolate plasma with absolute care to minimize damage of platelet.

Note:

- ○ Solid phase microplate consists of 12 detachable 8-well strips. Repack in the aluminum pouch unused strips together with desiccant and seal it for storage at 2 - 10°C.
- ○ While in use, Mouse HGF standard solution should be maintained at the ice cold water temperature and all reagents should be stored at 2 - 10°C after use. Samples should be stored frozen (-20°C or below).
- ○ Return the kit to 15 - 30°C before use.
- ○ As illustrated in Figs. 1 and 2, using 2 wells for each standard concentration, measure absorbance of Mouse HGF standard solutions in each assay (2 wells × 5 different standard concentration).



1) Adding samples

(1) High salt concentration samples (***Method A***)

If sample dilution is required, dilute with Sample diluent H before assay.

Add 50 µL each of Sample diluent H in the wells for the standard solution (10 wells) and add 50 µL of each Mouse HGF standard solution (concentration 0, 1.0, 3.0, 10, and 25 ng/mL), 2 wells for each concentration. Add 50 µL each of Sample diluent L in samples wells and add 50 µL each of samples, 2 wells for each sample.

(2) Low salt concentration samples (***Method B***)

If sample dilution is required, dilute with Sample diluent L before assay.

Add 50 µL each of Sample diluent L in all standard solution and samples wells. Add 50 µL of each Mouse HGF standard solution (concentration 0, 0.4, 1.0, 3.0, and 10 ng/mL) and samples in respective wells, 2 wells for each standard solution and sample.

2) 1st reaction

Cover the microplate surface with the plate seal supplied and leave the microplate to stand at 15 - 30°C for 20 hrs (16 - 24 hrs).

3) Washing

Remove the plate seal and suck out the well content with an aspirator.
Using a polyethylene washing bottle, fill wells with the washing solution prepared in 2) Washing solution of “2. Preparation of reagents”. Hold the microplate upside down and vigorously shake out the washing solution. After repeating this washing 5 times, gently tap the plate surface on paper towel to remove the washing solution.

Note: While washing the microplate wells, do not dry the inner wall of wells.
As soon as washing is over, immediately follow the next steps.

4) Addition of Anti-HGF rabbit antibody

Add 100 µL each of Anti-HGF rabbit antibody in all wells.

5) 2nd reaction

Cover the microplate surface with the plate seal supplied. Leave the microplate to stand at 15 - 30°C for 2 hrs.

6) Washing

Repeat the procedure 3) above to wash the microplate wells.

7) Addition of Enzyme labeled antibody

Add 100 µL each of Enzyme labeled antibody in all wells.

8) 3rd reaction

Cover the microplate surface with the plate seal supplied. Leave it to stand at 15 - 30°C for 2 hrs.

9) Preparation of Enzyme substrate solution (containing color developer)

15 min before the end of the 3rd reaction, prepare Enzyme substrate solution (containing color developer) according to the instruction in 1) Enzyme substrate solution (containing color developer) of “2. Preparation of reagents”.

10) Washing

Repeat the procedure 3) above to wash the microplate wells.

11) Addition of Enzyme substrate solution (containing color developer)

Add 100 μL each of Enzyme substrate solution (containing color developer) prepared in 9) above in all wells.

12) Enzyme reaction

Leave the microplate to stand at 15 - 30°C in the dark for 30 min.

13) Addition of the Reaction stopper solution

Add 50 μL each of Reaction stopper solution in all wells and thoroughly mix.

14) Absorbance measurement

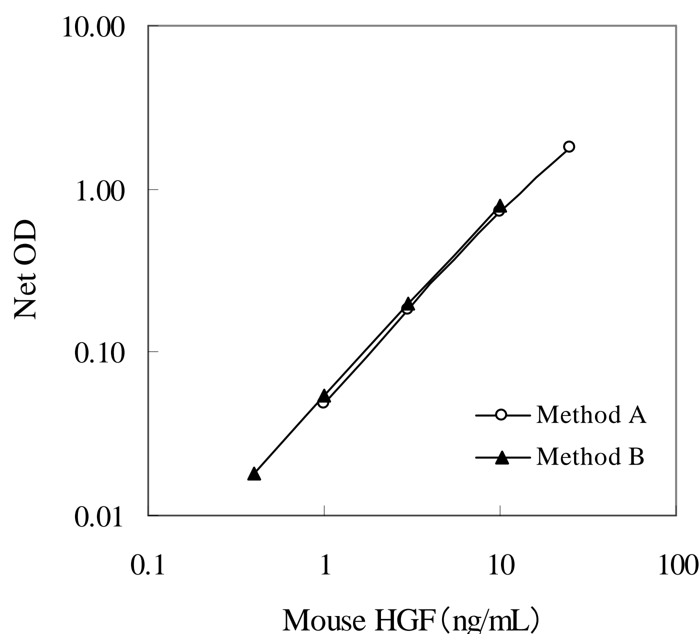
Measure on a microplate reader absorbance of each well (wavelength 492 nm or 490 nm). When a dual wavelength microplate reader is used, set the reference wavelength at 620 nm or longer. Absorbance must be measured within 2 hrs after stopping the enzyme reaction.

Determination

1. **Preparation of the calibration curve by Mouse HGF standard solution**

1) Calculate (mean absorbance of the respective concentration of Mouse HGF standard solution - mean absorbance of 0 ng/mL of Mouse HGF standard solution) (Net OD).

2) Plot on logarithmic scales Net OD of the respective concentration.



2. **Calculation of the mouse HGF in samples**
    1) When absorbance of samples exceeds that of maximum concentration of Mouse HGF standard solution, dilute samples and repeat the assay again.
    2) Calculate (mean absorbance of samples - mean absorbance of 0 ng/mL concentration of Mouse HGF standard solution) (Net OD).
    3) Apply the values calculated in 2) above to the calibration curve and determine mouse HGF concentration of samples.

Handling and operational precautions

1. **General precautions**
    1) Make sure to return the kit to the 15 - 30°C before use.
    2) Do not mix up kit components of different production lots. (Mouse HGF standard solution carries a different lot number from other components in the kit.)
    3) Assay strictly as instructed.
    4) Do not use expired reagents.
    5) Avoid contamination of the kit reagents with microorganisms.
    6) Thoroughly wash equipment used for the assay and rinse them with distilled water.
    7) Replace micropipette tips for each sample and reagent.
2. **Operational precautions**
    1) Measure absorbance of Mouse HGF standard solution for each assay.
    2) Once assay is started, complete it within the prescribed time and allow the same length of reaction time for Mouse HGF standard solution and samples.
    3) Make sure that all reactions take place at 15 - 30°C.
    4) Enzyme substrate solution (containing color developer) should be prepared 15 min before use and used up within 60 min after preparation.
    5) Measure absorbance within 2 hrs after stopping the enzyme reaction.
    6) Do not scrape the microplate or touch the bottom of wells. Do not dry up the inner wall of the wells during operation.
3. **Kit handling precautions**
    1) Avoid contact with skin of Enzyme substrate solution, Color developer and Reaction stopper solution. (They are toxic and irritable and may cause a burn.) Because Reaction stopper solution is strong acidity, make caution to handle and to discard it.
    2) When discarding the sample diluent, run sufficient volume of tap water as it contains 0.1% sodium azide.

Storage and shelf life

Store the kit at 2 - 10°C in the dark avoiding freezing. This kit is stable for 6 months after the date of manufacture.

Package

1 kit for 96 tests   Code No. 1Z85

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Assay Procedures and Well Arrangement

Method A - High salt concentration samples

		HGF standard	Samples
1	Well Arrangement	1A - 1H, 2A - 2B	2C - 12H
2	Addition of the HGF standard solutions and samples		
	Mouse HGF standard solution	50 µL	--
	Sample*	--	50 µL
	Sample diluent L	--	50 µL
	Sample diluent H	50 µL	--
3	1st reaction	20 hrs at 15 - 30°C	
4	Washing	5 times	
5	Addition of Anti-HGF rabbit antibody	100 µL	100 µL
6	2nd reaction	2 hrs at 15 - 30°C	
7	Washing	5 times	
8	Addition of Enzyme labeled antibody	100 µL	100 µL
9	Third reaction	2 hrs at 15 - 30°C	
10	Washing	5 times	
11	Addition of Enzyme substrate solution (containing color developer)	100 µL	100 µL
12	Enzyme reaction	30 min in the dark at 15 - 30°C	
13	Addition of Reaction stopper solution	50 µL	50 µL
14	Absorbance measurement	492 nm or 490 nm	
15	Determination		

*Sample or sample diluted with Sample diluent H.

Assay Procedures and Well Arrangement

Method B - Low salt concentration samples

		HGF standard	Samples
1	Well Arrangement	1A - 1H, 2A - 2B	2C - 12H
2	Addition of the HGF standard solutions and samples		
	Mouse HGF standard solution	50 µL	--
	Sample*	--	50 µL
	Sample diluent L	50 µ L	50 µL
3	1st reaction	20 hrs at 15 - 30°C	
4	Washing	5 times	
5	Addition of Anti-HGF rabbit antibody	100 µL	100 µL
6	2nd reaction	2 hrs at 15 - 30°C	
7	Washing	5 times	
8	Addition of Enzyme labeled antibody	100 µL	100 µL
9	Third reaction	2 hrs at 15 - 30°C	
10	Washing	5 times	
11	Addition of Enzyme substrate solution (containing color developer)	100 µL	100 µL
12	Enzyme reaction	30 min in the dark at 15 - 30°C	
13	Addition of Reaction stopper solution	50 µL	50 µL
14	Absorbance measurement	492 nm or 490 nm	
15	Determination		

*Sample or sample diluted with Sample diluent L.