HGF (Human)

ELISA KIT PROTOCOL

(Catalog No.: EK-012-64)
(range: 125 -8000 pg/ml)

PHOENIX PHARMACEUTICALS, INC.

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INTRODUCTION AND PROTOCOL OVERVIEW

Hepatocyte Growth Factor (HGF) is a secreted growth factor which consists of a 69-kDa alpha-chain and 34-kDa beta-chain. A disulfide bond between the alpha and beta chains produces the active, heterodimeric molecule. It is secreted by mesenchymal cells and targets and acts primarily upon epithelial cells and endothelial cells, but also acts on haemopoietic progenitor cells to be a paracrine factor for the cellular growth, motitlity. HGF plays key roles in the attenuation of disease progression as an intrinsic repair factor. It is also evident that HGF levels are regulated under different conditions, for example, during the course of pregnancy, aging, and disease.

Phoenix Pharmaceutical's HGF (Human) ELISA Kit is designed to measure the concentration of HGF from human serum/plasma, or conditioned medium.

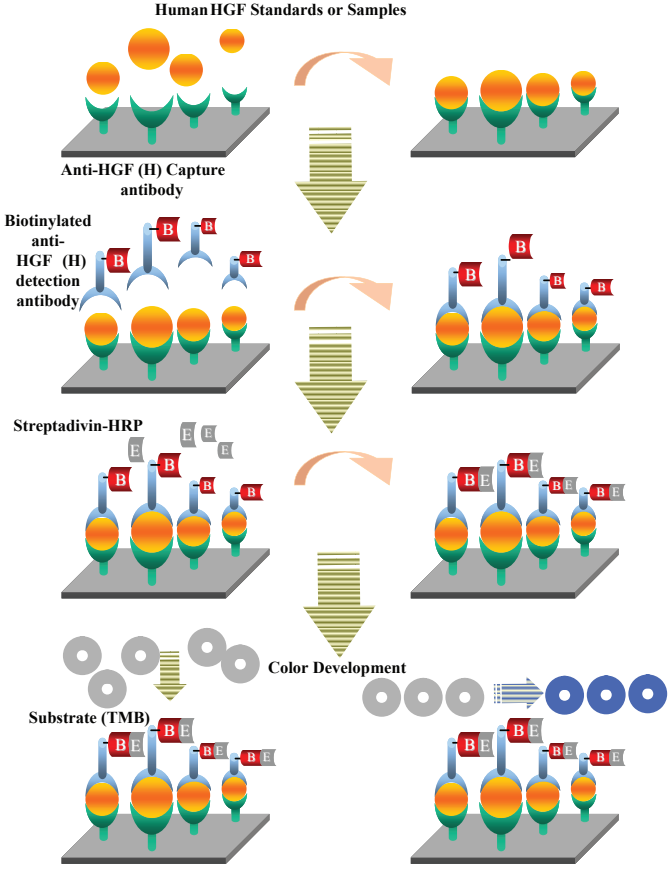
The immunoplate in this kit is precoated with anti-HGF Capture Antibody and the nonspecific binding sites are blocked. The Human HGF in the sample or in the standard solution can bind to the capture antibody immobilized in the wells. After washing procedure, the Biotinylated anti-HGF Detection Antibody which can bind to the HGF trapped in the wells is added. After washing, the Streptavidin-Horseradish Peroxidase (SA-HRP) which catalyzes the Substrate Solution (TMB) is added. The enzyme-substrate reaction is terminated by the addition of a stop solution. The intensity of the color is directly proportional to the amount of HGF in the standard solutions or samples. A standard curve of HGF with known concentration can be established accordingly. The HGF with unknown concentration in samples can be determined by extrapolation to this standard curve.

ASSAY CONDITIONS

Plasma, serum, culture media, tissue homogenate, CSF, urine or any biological fluid can be assayed as long as the level of the sample is high enough the for the sensitivity of the kit to detect it.

CAUTION: Phoenix Pharmaceuticals guarantees that its products conform to the information contained in this publication. The purchaser must determine the suitability of the product for its particular use and establish optimum sample concentrations.

Assay Principle



LIST OF COMPONENTS

Store all components at 4°C. DO NOT FREEZE.

1. Wash Buffer Concentrate *(20x, 50ml)*.....**Catalog No. EK-BUF**
2. Assay Diluent (PBS and BSA) .....**Catalog No. EK-BUF-012-64** *(1x, 60ml)*
3. 96 Well anti-HGF Capture.....**Catalog No. EK-Plate-012-64** Antibody-Coated Plate *(1 plate)*
4. HGF (Human) Standard.....**Catalog No. EK-S-012-64** *(lyophilized powder, 16 ng/vial)*
5. Biotinylated anti-HGF.....**Catalog No. EK-D-012-64** Detection Antibody *(1 vial)*
6. HGF (Human) Positive Control.....**Catalog No. EK-PC-012-64** *(1 vial)*
7. Streptavidin-Horseradish Peroxidase.....**Catalog No. EK-HRP** (SA-HRP) *(6000x, 15µl)*
8. Substrate Solution (TMB) *(12ml)*.....**Catalog No. EK-SS**
9. Stop Solution 2N HCl *(15ml)* .....**Catalog No. EK-HCL**
10. Acetate Plate Sealer (APS) *(3 pieces)*.....**Catalog No. EK-APS**
11. Assay Diagram *(1 sheet)*

MATERIALS REQUIRED BUT NOT SUPPLIED

- Micropipettor(s) and disposable pipette tips
- Multi-channel pipette capable of dispensing 50-100µl
- Solution Reservoir *(recommended)*
- Microtiter plate washer *(recommended)*
- Orbital plate shaker capable of 300-500 rpm *(recommended)*
- Microtiter plate reader capable of absorbance measurement 450nm
- Well-closed containers (15ml tubes or more in capacity)
- Absorbent material for blotting

REAGENT PREPARATION

Note: *The kit should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay. It is highly recommended that the solutions be used as soon as possible after rehydration.*

1. **1x Wash Buffer:** Dilute the **20x** Wash Buffer Concentrate with 950ml of distilled water. If crystals are observed in the **20x** Wash Buffer, warm the bottle in a 37°C water bath for approximately 30 minutes or until the crystals disappear. After preparation, store **1x** Wash Buffer at 4°C. This buffer is used to wash the plate wells.
2. **Biotinylated anti-HGF (Human) Detection Antibody:** Rehydrate biotinylated anti-HGF (Human) Detection Antibody with 100µl of **1x** Assay Diluent, vortex (centrifuge the tube to dislodge powder from the cap or walls). Further dilute stock solution of biotinylated anti-HGF (Human) Detection Antibody to 1:100 as needed with Assay Diluent and mix thoroughly before use.
3. **Streptavidin-Horseradish Peroxidase (SA-HRP):** Centrifuge the HRP vial (15µl) provided in this kit (3,000-5,000 rpm, 5 seconds) and dilute HRP with **1x** Assay Diluent to 1:6000 before use. Vortex thoroughly.
4. **Human HGF Positive Control:** Rehydrate Human HGF Positive Control with 600µl of **1x** Assay diluent (centrifuge the tube to dislodge powder from cap or walls). Vortex thoroughly.

SAMPLES PREPARATION

(A) Prepare "2x of sample diluent" for the assay wells by dilution of a volume of sample (for example: 500 µl of serum or plasma) with the same volume of Assay Diluent. In the case of expecting concentration of "2x sample diluents" is higher than the 8ng/ml, it is recommend to do further dilution with Assay Diluent to be 4x or 8x sample diluents for such assay.

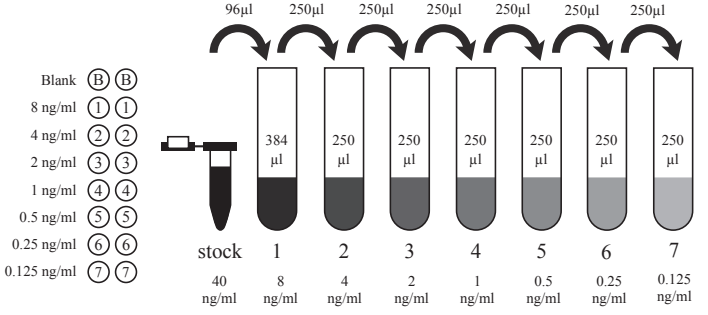
(B) All the samples after dilution must be applied to the plate as soon as possible to minimize proteolysis of sample proteins. The samples of plasma, serum, culture media, and tissue homogenate can be assayed.

HUMAN HGF STANDARD PREPARATION

1. Rehydrate recombinant Human HGF Standard (16 ng) with 400µl **1x** Assay Diluent, vortex. Allow the solution to sit at least 10 minutes at room temperature (20-23°C) to completely dissolve in solution. Vortex and centrifuge before use. The concentration of this stock solution is 40 ng/ml.

2. Prepare Human HGF standard solutions as follows:

Standard No.	Std. volume	Assay Buffer	Concentrations
Stock	Powder	400µl	40 ng/ml
#1	96µl of Stock	384µl	8 ng/ml
#2	250µl of #1	250µl	4 ng/ml
#3	250µl of #2	250µl	2 ng/ml
#4	250µl of #3	250µl	1 ng/ml
#5	250µl of #4	250µl	0.5 ng/ml
#6	250µl of #5	250µl	0.25 ng/ml
#7	250µl of #6	250µl	0.125 ng/ml



HGF (Human) ELISA PROTOCOL

1. Thoroughly read this protocol before performing an assay. Allow all reagents to come to room temperature (20-23°C) prior to the start of the assay (25-45 mins).
2. Remove Capture Antibody-Coated Plate from its zip-lock foil pouch. Remove unneeded strips from the plate frame, reseal them in the foil pouch, and return the foil pouch to 4°*C*.
3. Leave wells A-1 and A-2 empty as **Blank**.
4. Add 100µl of the prepared Human HGF Standard solutions from **#7** to **#1** (reverse order of serial dilution) in duplicate to each well.
5. Add 100µl of Human HGF Positive Control solution in duplicate.
6. Add 100µl diluted samples in duplicate into their designated wells.

7. Seal the immunoplate with Acetate Plate Sealer (APS). Incubate for 2 hours at room temperature (20-30°C) on a plate shaker (300-400 rpm).

8. Before washing the plate, remove the plate sealer carefully. Completely discard the liquid from wells. Wash each well with 300-350µl Wash Buffer four times. At the end of each the wash, discard the buffer, invert the plate, and tap on a clean absorbent towel.

9. Add 100µl diluted biotinylated anti-Human HGF Detection Antibody into each well except the **Blank** well. Reseal the immunoplate with plate sealer and incubate for 1 hours at room temperature on a plate shaker (300-400rpm).

10. Remove APS from the plate. Wash 4 times with the **1x** Wash Buffer as described in step 8.

11. Add 100µl diluted SA-HRP solution into each well. Reseal the immunoplate with plate sealer and incubate the plate for 30 mins at room temperature on plate shaker (300-400rpm).

12. Repeat step 10.

13. Add 100µl Substrate Solution (TMB) into each well. Reseal the plate with plate sealer to protect from light and incubate the plate for 15-30 minutes at room temperature on plate shaker (300-400 rpm). After incubation, deep blue coloring should be observed in the 8 ng/ml standard wells. The incubation should stop immediately if the middle concentration of standard wells (1 ng/ml) turn to blue color.

14. Add 100µl Stop Solution (2N Hydrochloric Acid) into each well to stop the reaction. The color in the well should change from blue to yellow. If the color change does not appear to be uniform, gently tap the plate to ensure thorough mixing. Proceed to the next step within 20 minutes.

15. Remove APS. Read Absorbance O.D. at 450nm using a Microtiter Plate
Reader.

ADDITIONAL RECOMMENDED PROCEDURAL NOTES:

- Reagents of different lot numbers should not be mixed.
- Recheck the reagent labels when loading the plate to ensure that everything is added correctly.
- Unused microplate strips should be placed in the foil pouch with a desiccant and stored at 4°C. Do not allow moisture to enter the wells.
- When handling the plate, avoid touching the bottom.
- Manual washing may cause high duplicate coefficient variations. To reduce this factor, liquid from the plate should be removed by inverting and blotting the plate on an absorbent material.
- If the room temperature is not within the suggested range (20-23°C), variations in results may occur.
- The same reservoir for the reagents may be reused if the reservoir is washed well with distilled water before each use.
- Each laboratory must determine the appropriate dilution factors for the samples to be measured to ensure that the samples are within the dynamic range of the standard curve.
- High levels of interfering proteins may cause variations within the sample results, therefore, it is imperative to select the appropriate sample preparation procedure to obtain the optimal results.
- Each time a new tip is used, make sure the tip is secure and free of air bubbles. For better intra-assay variation, aspirate and expel a reagent or sample back into the container a few times prior to loading.
- Avoid submerging the whole tip into reagents because droplets can accumulate at the end of the tip causing an excess of reagent to be loaded into the well. This can lead to poor results.
- For optimal results, an orbital plate shaker capable of 300-500 rpm is recommended for all incubations.
- Modification of the existing protocol (i.e. standard dilutions, pipetting technique, washing technique, incubation time or temperature, storage conditions, and kit expiration) may affect the sensitivity and specificity of the test.

SUMMARY OF ASSAY PROTOCOL

Add 100µl/well of Human HGF Standard, Positive Control or Samples

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Incubate at room temperature for 2 hours

▼

Wash immunoplate 4 times with 350µl/well of **1x** Wash Buffer

▼

Add 100µl/well of Biotinylated Anti-Human HGF Detection Antibody

▼

Incubate at room temperature for 1 hour

▼

Wash immunoplate 4 times with 350µl/well of **1x** Wash Buffer

▼

Add 100µl/well of SA-HRP solution

▼

Incubate at room temperature for 30 minutes

▼

Wash immunoplate 4 times with 350µl/well of **1x** Wash Buffer

▼

Add 100µl/well of Substrate Solution (TMB)

▼

Incubate at room temperature (20-23°C) for 15-30 mins

▼

Terminate reaction with 100µl/well of 2N HCl

▼

Read absorbance O.D. at 450nm and calculate results

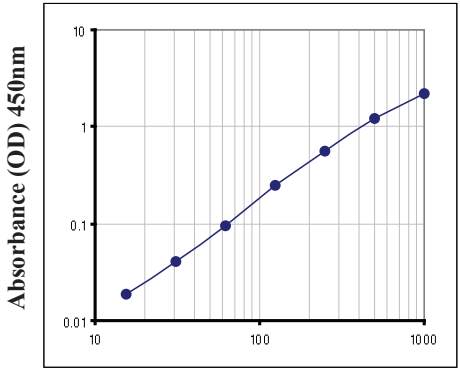
CALCULATION OF RESULTS

Plot the standard curve on log-log graph paper. Known concentration of Human HGF Standard and its corresponding O.D. reading is plotted on the log scale (X-axis) and the log scale (Y-axis), respectively. The standard curve shows a correlated relationship between Human HGF concentrations and the corresponding O.D. absorbance. As the standard concentration increases, the intensity of the yellow color, and in turn the O.D. absorbance, increases.

The concentration of Human HGF within a sample is determined by plotting the sample's O.D. on the Y-axis, then drawing a horizontal line to intersect with the standard curve. A vertical line dropped from this point will intersect the X-axis at a coordinate corresponding to the Human HGF concentration in the unknown sample.

Refer to QC Data sheet for acceptable values of the positive control.

Human HGF Standard Curve



STORAGE

1. Store the kit at 4°C upon receipt. The kit should be equilibrated to room temperature (20-23°C) before assay.
2. Store **1x** Assay Buffer at 4°C.
3. Remove any unneeded strips from Human HGF antibody-coated plate, reseal them in zip-lock foil and keep at 4°C.
4. Keep rehydrated solution of Human HGF Standard, Biotinylated anti-Human HGF Detection Antibody at -20°C and SA-HRP at 4°C. Prepare only the required amount.

NOTE:

1. It is recommended that the solutions be used on the same day of rehydration.
2. After adding Stop Solution, read the plate within 20 minutes.

REFERENCES

1. Funakoshi H, Nakamura T (2003). "Hepatocyte growth factor: from diagnosis to clinical applications". Clin. Chim. Acta 327 (1–2): 1–23. doi:10.1016/S0009-8981(02)00302-9. PMID 12482615.

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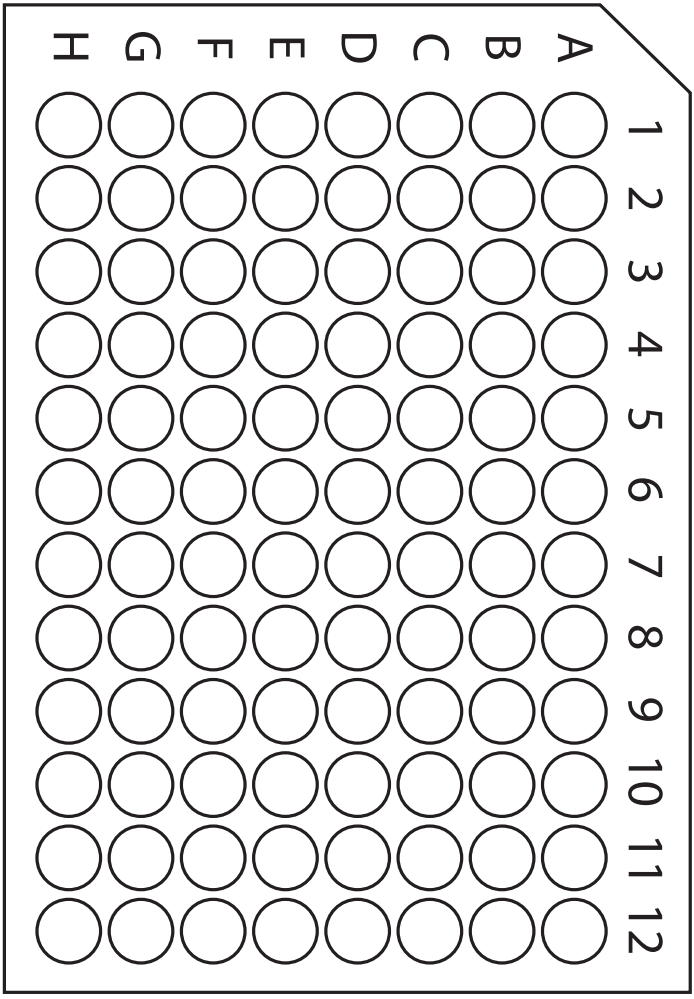
ASSAY DIAGRAM



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