HGF (Human) ELISA Kit

Catalog Number KA0343

96 assays

Version: 10

Intended for research use only

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Introduction

Intended Use

For quantitative of Human HGF concentrations in cell culture supernates, serum and plasma (EDTA, citrate).

Background

Hepatocyte growth factor (HGF) is the most potent mitogen for mature parenchymal hepatocytes in primary culture, and seems to be a hepatotrophic factor that acts as a trigger for liver regeneration after partial hepatectomy and liver injury. HGF has a relative molecular mass (Mr) of 82,000 and is a heterodimer composed of a large alpha-subunit of Mr 69,000 and a small beta-subunit of Mr 34,000. The protein consists of 728 amino acid residues, including a possible signal peptide at the N-terminus. HGF may serve as a paracrine mediator to control placental development and growth. This growth factor may play an important role as a paracrine mediator of the proliferation of melanocytes and endothelial cells, as well as cells of epithelial origin. The gene encoding the human HGF is assigned to human chromosome 7.

Principle of the Assay

The HGF (Human) ELISA Kit is a solid phase immunoassay specially designed to measure Human HGF with a 96-well strip plate that is pre-coated with antibody specific for HGF. The detection antibody is a biotinylated antibody specific for HGF. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Human HGF with immunogen: Expression system for standard: sf21; Immunogen sequence: Q32-R494 (alpha) & V495-S728 (beta). The kit is analytically validated with ready to use reagents.

To measure Human HGF, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Human HGF in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human HGF in the sample.

General Information

Materials Supplied

List of component

Component	Amount
Anti-Human HGF Pre-coated 96-well plate strip microplate	96 (8x12) wells
Human HGF standard	10 ng/tube x 2
Human HGF Biotinylated antibody (100x)	130 µL
Avidin-Biotin-Peroxidase Complex (100x)	130 µL
Sample diluent	30 mL
Antibody diluent	12 mL
Avidin-Biotin-Peroxidase Diluent	12 mL
Color developing agent (TMB)	10 mL
Stop solution	10 mL
Wash Buffer Powder	1 pack
Adhesive cover	4 slides

Storage Instruction

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles.

Materials Required but Not Supplied

- ✓ Microplate Reader capable of reading absorbance at 450 nm.
- ✓ Automated plate washer (optional).
- ✓ Pipettes and pipette tips capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- ✓ Multichannel pipettes are recommended for large amount of samples.
- ✓ Deionized or distilled water.
- ✓ 500 mL graduated cylinders.
- ✓ Test tubes for dilution.

Precautions for Use

This protocol must be read in its entirety before using this product. For research use only. Not for use in diagnostic procedures.

- ✓ Notice Before Application

  Please read the following instructions before starting the experiment.

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don’t let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don’t reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

Assay Protocol

Reagent Preparation

- ✓ Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-25 min) is based on 37°C.
  - Wash buffer
    Dissolve the wash buffer powder in 1000 mL of water to make 1X PBS wash buffer.
  - Biotinylated Anti-Human HGF antibody
    It is recommended to prepare this reagent immediately prior to use by diluting the Human HGF Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µL by adding 1 µL of Biotinylated antibody (100x) to 99 µL of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
  - Avidin-Biotin-Peroxidase Complex
    It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µL by adding 1 µL of Avidin-Biotin-Peroxidase Complex (100x) to 99 µL of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
  - Human HGF Standard
    It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Human HGF standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/mL using 1 mL of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
  - Microplate
    The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.
- ✓ Dilution of Human HGF Standard
  1. Number tubes 1-8. Final Concentrations to be Tube # 1 – 8000 pg/mL, #2 – 4000 pg/mL, #3 – 2000 pg/mL, #4 – 1000 pg/mL, #5 – 500 pg/mL, #6 – 250 pg/mL, #7 – 125 pg/mL, #8 – Sample Diluent serves as the zero standard (0 pg/mL).
  2. To generate standard #1, add 800 µL of the reconstituted standard stock solution of 10 ng/mL and 200 µL of sample dilution to tube #1 for a final volume of 1000 µL. Mix thoroughly.
  3. Add 300 µL of sample diluent to tubes # 2-7.
  4. To generate standard #2, add 300 µL of standard #1 from tube #1 to tube #2 for a final volume of 600 µL. Mix thoroughly.
  5. To generate standard #3, add 300 µL of standard #2 from tube #2 to tube #3 for a final volume of 600

µL. Mix thoroughly.

6. Continue the serial dilution for tube #4-7.

Sample Preparation

✓ Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

- Cell culture supernatants: Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
- Serum: Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
- Plasma: Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C.

  **Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.*

✓ Sample Dilution Guideline

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µL of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

Assay Procedure

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Reagent Preparation if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µL of the standard, samples, or control per well. Add 100 µL of the sample diluent buffer into the zero well. At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 minutes at 37°C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µL of the prepared 1x Biotinylated Anti-Human HGF antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.

a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

b. Add 300 µL of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

c. Repeat steps a-b 2 additional times.

9. Add 100 µL of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.

    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

    b. Add 300 µL of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

    c. Repeat steps a-b 4 additional times.
11. Add 90 µL of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100 µL of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450 nm.

Data Analysis

Calculation of Results

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit.

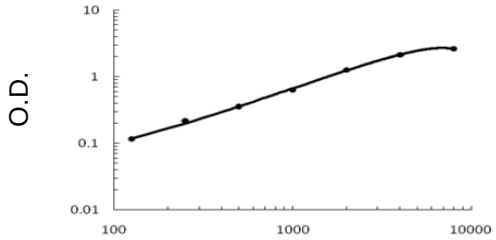
Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

Note: For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

✓ The HGF (Human) ELISA Kit Standard Curve example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/mL)	0	125	250	500	1000	2000	4000	8000
O.D.	0.014	0.116	0.217	0.357	0.631	1.261	2.133	2.604



Concentration (pg/mL)

A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Performance Characteristics

✓ Detection Range: 125 pg/mL-8000 pg/mL

✓ Sensitivity: < 5 pg/mL

* The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.

✓ Specificity: Nature and recombinant Human HGF

✓ Cross-reactivity: There is no detectable cross-reactivity with other relevant proteins.

✓ Intra/Inter Assay Variability

- Intra-Assay Precision (Precision within an assay)

  Three samples of known concentration were tested on one plate to assess intra-assay precision.

- Inter-Assay Precision (Precision across assays)

  Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean (pg/mL)	169	1246	4183	168	1364	4239
Standard deviation	9.8	87.22	267.71	9.91	118.66	313.68
CV (%)	5.8%	7%	6.4%	5.9%	8.7%	7.4%

✓ Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/mL)	Lot2 (pg/mL)	Lot3 (pg/mL)	Lot4 (pg/mL)	Mean (pg/mL)	Standard Deviation	CV (%)
Sample 1	169	159	165	152	161	6.41	3.9%
Sample 2	1246	1103	1132	1240	1180	63.61	5.3%
Sample 3	4183	3905	4308	3657	4013	252.15	6.2%

*number of samples for each test n=16.

Resources

Plate Layout

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H