Hgf (Mouse) ELISA Kit

Catalog Number KA1786

96 assays

Version: 03

Intended for research use only

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Introduction

Background

Hgf (Mouse) ELISA Kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of mouse HGF cell lysate and tissue lysate. This assay employs an antibody specific for mouse HGF coated on a 96-well plate. Standards and samples are pipetted into the wells and HGF present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse HGF antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of HGF bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm

General Information

Materials Supplied

Component	Amount
HGF Microplate (Item A): coated with anti-mouse HGF	96(8x12) wells
Wash Buffer Concentrate (20x) (Item B): 20x concentrated solution	25 ml
Standards (Item C): recombinant mouse HGF	2 vials
Sample Diluent Buffer (Item D): 5x concentrated buffer. For Standard/Sample (cell lysate/tissue lysate) diluent	10 ml
Assay Diluent (Item E): 5x concentrated buffer. For Detection Antibody (Item F) and HRP-Streptavidin concentrate (Item G) diluent	15 ml
Detection Antibody HGF (Item F): biotinylated anti-mouse HGF (each vial is enough to assay half microplate)	2 vials
HRP-Streptavidin concentrates (Item G): 500x concentrated HRP-conjugated Streptavidin	200 µl
TMB One-Step Substrate Reagent (Item H): of 3, 3', 5, 5'-tetramethylbenzidine (TMB) in buffered solution	12 ml
Stop Solution (Item I): 0.2 M sulfuric acid	8 ml
Cell lysate buffer (Item J): 2x cell lysate buffer	5 ml

Storage Instruction

May be stored for up to 6 months at 2 to 8°C from the date of shipment. Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution. Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Note: the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Precision pipettes to deliver 2 µl to 1 ml volumes.
- ✓ Adjustable 1-25 ml pipettes for reagent preparation.
- ✓ 100 ml and 1 liter graduated cylinders.
- ✓ Absorbent paper.
- ✓ Distilled or deionized water.
- ✓ Log-log graph paper or computer and software for ELISA data analysis.
- ✓ Tubes to prepare standard or sample dilutions.

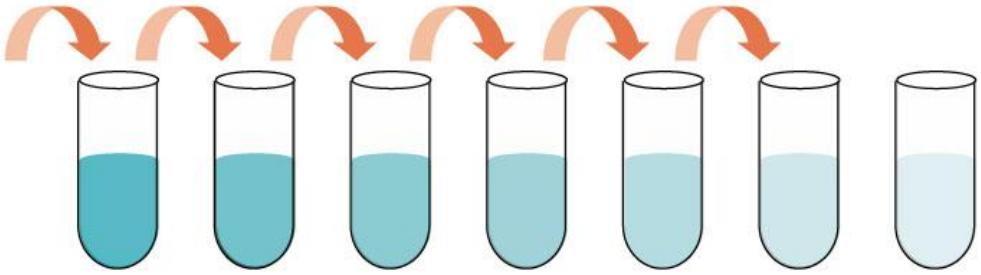
Assay Protocol

Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Sample dilution: Tissue lysate and cell lysate sample should be diluted at least 5-fold with 1 x Sample Diluent Buffer.
3. Sample Diluent Buffer (Item D) and Assay Diluent (Item E) should be diluted 5-fold with deionized or distilled water before use.
4. Preparation of standard: Briefly spin the vial of Item C and then add 400 µl 1x Sample Diluent Buffer into Item C vial to prepare a 60 ng/ml standard. Dissolve the powder thoroughly by a gentle mix. Pipette 240 µl Sample Diluent Buffer into each tube. Use the 60 ng/ml standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. Sample Diluent Buffer serves as the zero standard (0 ng/ml).

   Item C vial

   +400 µl 160 µl 160 µl 160 µl 160 µl 160 µl



   60 ng/ml 24 ng/ml 9.6 ng/ml 3.84 ng/ml 1.536 ng/ml 0.614 ng/ml 0 ng/ml
5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.
6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µl of 1x Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent and used in step 4 of Assay Procedure.
7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 500-fold with 1x Assay Diluent.

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 20 µl of HRP-Streptavidin concentrate into a tube with 10 ml 1x Assay Diluent to prepare a 500-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.
8. Cell lysate buffer should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).

Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard (see Reagent Preparation step 2) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.
   We recommend using 50-500 µg/ml of total protein for lysate sample. The amount of sample used depends on the abundance of target protein. More of the sample can be used if signals are too weak. If signals are too strong, the sample can be diluted further.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of 1x prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µl of Stop Solution (Item I) to each well. Read at 450 nm immediately

Summary

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4°C.
3. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately

Data Analysis

Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.

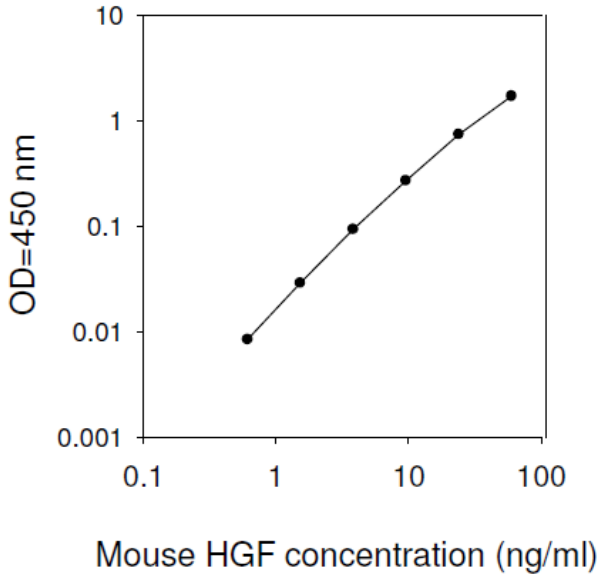


Figure 1: Typical Standard Curve for Hgf (Mouse) ELISA Kit.

Performance Characteristics

- Sensitivity

  The minimum detectable dose of HGH is typically less than 400 pg/ml.

- Recovery

  Recovery was determined by spiking various levels of mouse HGF into mouse tissue lysate and cell lysate. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Tissue lysate	120.2	110-130
Cell lysate	114.4	100-134

- LInearity

Sample Type		Tissue Lysate	Cell Lysate
1:2	Average % of Expected	118.9	139.4
	Range (%)	109-125	130-143
1:4	Average % of Expected	132.4	129.2
	Range (%)	120-144	120-137

- Reproducibility

  Intra-Assay: CV<10%

  Inter-Assay: CV<12%

- Specificity

  Cross Reactivity: This ELISA kit shows no cross-reactivity with the following cytokines tested: Mouse CD30, L CD30, T CD40, CRG-2, CTACK, CXCL16, Eotaxin, Eotaxin-2, Fas Ligand, Fractalkine, GCSF, GM-CFS, IFN-γ, IGFBP-3, IGFBP-5, IGFBP-6, IL-1α, IL-1β, IL-2, IL-3, IL-3 Rb, IL- 4, IL-5, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17, KC, Leptin R, LEPTIN(OB), LIX, L-Selectin, Lymphotactin, MCP-1, MCP-5, M-CSF, MIG, MIP-1α, MIP-1γ, MIP-2, MIP-3β, MIP-3α, PF-4, P-Selectin, RANTES, SCF, SDF-1α, TARC, TCA-3, TECK, TIMP-1, TNF-α, TNF RI, TNF RII, TPO, VCAM-1, VEGF

Resources

Troubleshooting

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting 2. Improper standard dilution	1. Check pipettes 2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1. Too brief incubation times 2. Inadequate reagent volumes or improper dilution	1. Ensure sufficient incubation time; assay procedure step 2 may change to over night 2. Check pipettes and ensure correct preparation
3. Large CV	1. Inaccurate pipetting	1. Check pipettes
4. High background	1. Plate is insufficiently washed 2. Contaminated wash buffer	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed. 2. Make fresh wash buffer
5. Low sensitivity	1. Improper storage of the ELISA kit 2. Stop solution	1. Store your standard at <-20°C after reconstitution, others at 4°C. Keep substrate solution protected from light 2. Stop solution should be added to each well before measure

Plate Layout

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9								
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7								
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5								
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3								
2								
1								
	A	B	C	D	E	F	G	H