ELISA Kit

Catalog # KAC2211 (96 tests)

Human

HGF

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Table of Contents

Contents and Storage

Storage

Store at 2 to 8°C.

Contents

Reagents Provided	96 Test Kit
Hu HGF Standard, lyophilized, recombinant Hu HGF. Refer to vial label for quantity and reconstitution volume.	2 vials
Hu HGF Control, recombinant Hu HGF lyophilized. Refer to vial label for quantity and reconstitution volume. Once reconstituted, aliquot and store at -20°C or below. Avoid repeated freeze thaw cycles.	1 vial
Standard Diluent Buffer. Contains 0.1% sodium azide; 25 mL per bottle.	1 bottle
Incubation Buffer. Contains 0.1% sodium azide; 25 mL per bottle.	1 bottle
Hu HGF Antibody-Coated Wells, 96 wells per plate.	1 plate
Hu HGF Biotin Conjugate (Biotin-labeled anti-HGF). Contains 0.1% sodium azide; 11 mL per bottle.	1 bottle
Streptavidin-Peroxidase (HRP), (100x) concentrate. Contains 2.5 mM thymol; 0.125 mL per vial.	1 vial
Streptavidin-Peroxidase (HRP) Diluent. Contains 2.5 mM thymol and 0.05% Proclin® 300; 25 mL per bottle.	1 bottle
Wash Buffer Concentrate (25X); 100 mL per bottle.	1 bottle
Stabilized Chromogen, *Tetramethylbenzidine (TMB);* 25 mL per bottle.	1 bottle
Stop Solution; 25 mL per bottle.	1 bottle
Plate Covers, adhesive strips.	3

Disposal Note

This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Proclin® 300 is toxic. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

Safety

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

Introduction

Purpose

The Invitrogen Human Hepatocyte Growth Factor (Hu HGF) ELISA is to be used for the quantitative determination of Hu HGF in human serum, EDTA plasma, buffered solution, or cell culture medium. Heparinized plasma samples are not recommended in this assay because of poor recoveries (50%).

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Principle of the Method

The Invitrogen Hu HGF kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Hu HGF has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Hu HGF content, control specimens, and unknowns, are pipetted into these wells.

During the first incubation, the Hu HGF antigen binds to the immobilized (capture) antibody on one site. After washing, a biotinylated monoclonal antibody specific for Hu HGF is added. During the second incubation, this antibody binds to the immobilized Hu HGF captured during the first incubation.

After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a second incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Hu HGF present in the original specimen.

Background Information

Hepatocyte Growth Factor (HGF), also called Scatter Factor, consists of two subunits held together by a disulfide bond. The alpha subunit (69 kDa) and the beta subunit (34 kDa) have a length of 440 amino acids and 234 amino acids, respectively.

HGF is a multifunctional cytokine that acts as a mitogen, a motogen and a morphogen, explaining its functions in organogenesis and tissue regeneration (1). HGF is constitutively produced by bone marrow stromal cells and enhances hematopoiesis (4).

Methods

Materials Needed But Not Provided

- Microtiter plate reader (at or near 450 nm) with software
- Calibrated adjustable precision pipettes
- Distilled or deionized water
- Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.)
- Glass or plastic tubes for diluting solutions
- Absorbent paper towels
- Calibrated beakers and graduated cylinders

Procedural Notes

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
8. **Do not mix or interchange different reagent lots from various kit lots**.
9. Do not use reagents after the kit expiration date.
10. Absorbances should be read immediately, but can be read up to 2 hours after assay completion. For best results, keep plate covered in the dark.
11. In-house controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
12. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
13. Because Stabilized *Chromogen* is light sensitive, avoid prolonged exposure to light. Avoid contact between chromogen and metal, or color may develop.

Directions for Washing

- **Incomplete washing will adversely affect the test outcome**. All washing must be performed with the *Wash Buffer* provided.
- Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip into the bottom of each well. Take care not to scratch the inside of the well. After aspiration, fill the wells with at least 0.4 ml of diluted *Wash Buffer*. Let soak for 15 to 30 seconds, and then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- Alternatively, the diluted *Wash Buffer* may be put into a squirt bottle. If a squirt bottle is used, flood the plate with the diluted *Wash Buffer*, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
- If using an automated washer, follow the washing instructions carefully.

Preparation of Reagents

Dilution of Standard

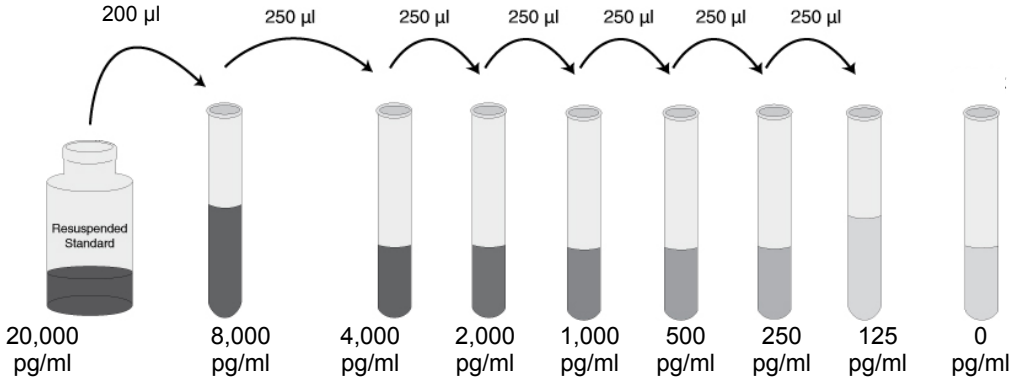
Note: Either glass or plastic tubes may be used for standard dilutions.

This assay has been calibrated against a highly purified recombinant human pro-HGF. Use the equation below to convert the measured sample values to equivalent NIBSC 95/556 International Units. NIBSC (95/556) equivalent value (mIU/ml) = 0.3 x Invitrogen HGF ELISA value (pg/ml).

1. Reconstitute standard to 20,000 pg/ml with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and **allow to sit for 20 minutes** to ensure complete reconstitution. Use standard within 1 hour of reconstitution.
2. Add 0.200 ml of the reconstituted standard to a tube containing 0.300 ml *Standard Diluent Buffer*. Label as 8,000 pg/ml Hu HGF. Mix.
3. Add 0.250 ml of *Standard Diluent Buffer* to each of 6 tubes labeled 4,000, 2,000, 1,000, 500, 250 and 125 pg/ml Hu HGF.
4. Make serial dilutions of the standard as described in the following dilution diagram. Mix thoroughly between steps.

Note

Remaining reconstituted standard should be discarded. Return the *Standard Diluent Buffer* to the refrigerator.



Preparing SAV-HRP

Note: Prepare within 15 minutes of usage. The *Streptavidin-HRP* (100x concentrate) is in 50% glycerol, which is viscous. To ensure accurate dilution, allow *Streptavidin-HRP* concentrate to reach room temperature. Gently mix. Pipette *Streptavidin-HRP* concentrate slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Dilute 10 µl of this 100x concentrated solution with 1 ml of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.
2. Return the unused *Streptavidin-HRP* concentrate to the refrigerator.

# of 8-Well Strips	Volume of Streptavidin-HRP Concentrate	Volume of Diluent
2	20 µl solution	2 ml
4	40 µl solution	4 ml
6	60 µl solution	6 ml
8	80 µl solution	8 ml
10	100 µl solution	10 ml
12	120 µl solution	12 ml

Dilution of Wash Buffer

1. Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 ml may be diluted up to 1.25 liters, 100 ml may be diluted up to 2.5 liters). Label as Working Wash Buffer.
2. Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

Assay Procedure

Be sure to read the *Procedural Notes* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 150 µl of the *Incubation Buffer* to each well. Well(s) reserved for chromogen blank should be left empty.
3. Add 50 µl of the *Standard Diluent Buffer* to the zero standard wells.
4. Add 50 µl of standards, samples or controls to the appropriate microtiter wells. (See **Preparation of Reagents**.) Tap gently on the side of the plate to mix.
5. Cover plate with *plate cover* and incubate for **3 hours at room temperature.**
6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
7. Pipette 100 µl of biotinylated anti-Hu HGF *(Biotin Conjugate)* solution to each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
8. Cover plate with *plate cover* and incubate for **1 hour at room temperature.**
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
10. Add 100 µl Streptavidin-HRP Working Solution to each well except the chromogen blank(s). See **Preparation of Reagents**.
11. Cover plate with *plate cover* and incubate for **30 minutes at room temperature.**
12. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **Directions for Washing**.
13. Add 100 µl of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
14. Incubate for **30 minutes at room temperature and in the dark**. ***Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
15. Add 100 µl of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.

16. Read the absorbance of each well at 450 nm after having blanked the plate reader against a chromogen blank composed of 100 µl each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 2 hours after adding the *Stop Solution*.
17. Use a curve fitting software to generate the standard curve. A four parameter algorithm provides the best standard curve fit.
18. Read the concentrations for unknown samples and controls from the standard curve. Serum/plasma samples producing signals greater than that of the highest standard should be diluted in *Standard Diluent Buffer;* while cell culture samples should be diluted with the cell culture medium. The measured concentrations should be multiplied by the appropriate dilution factor.

Typical Data (Example)

The following data were obtained for the various standards over the range of 0 to 8,000 pg/ml Hu HGF.

Standard Hu HGF (pg/ml)	Optical Density (450 nm)
8,000	3.10
4,000	2.10
2,000	1.23
1,000	0.69
500	0.41
250	0.27
125	0.20
0	0.11

Performance Characteristics

Sensitivity

The minimum detectable dose of Hu HGF is 20 pg/ml. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

Precision

1. Intra-Assay Precision

Samples of known Hu HGF concentration were assayed in replicates of 24 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/ml)	307	1,271	3,210
SD	15	42	200
%CV	4.9	3.3	6.2

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 24 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/ml)	311	1,187	3,398
SD	21	54	161
%CV	7.0	4.5	4.7

SD = Standard Deviation
CV = Coefficient of Variation

Linearity of Dilution

Human serum and cell culture supernatant containing Hu HGF were serially diluted over the range of the assay in *Standard Diluent Buffer* or RPMI containing 10% fetal bovine serum, respectively. Linear regression analysis of samples versus the expected concentration yielded an average correlation coefficient of 0.99.

	Serum			Cell Culture		
Dilution	Measured (pg/ml)	Expected (pg/ml)	% Expected	Measured (pg/ml)	Expected (pg/ml)	% Expected
neat	6679	-	-	5674	-	-
1/2	3368	3340	101	2784	2837	98
1/4	1668	1670	100	1564	1419	110
1/8	910	835	109	713	709	100
1/16	414	417	99	383	355	108
1/32	234	209	112	191	177	108

Recovery

The recovery of Hu HGF added to human serum and EDTA plasma averaged 94% and 102%, respectively. The recovery of Hu HGF added to tissue culture medium containing 10% fetal bovine serum averaged 96%.

Specificity

Buffered solutions of a panel of substances at 400 ng/ml were assayed with the Invitrogen Hu HGF kit. The following substances were tested and found to have no cross-reactivity: human IL-1ra, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-12, IFN-α, IFN-γ, GM-CSF, OSM, MIP-1α, MIP-1β, LIF, MCP-1, TGF-β, EGF, VEGF, GRO-α, and RANTES.

Expected Values

Serum / Plasma

Each laboratory must establish its own values. For guidance, the mean of 19 normal sera was 1283 pg/ml (range: 475 to 3083 pg/ml). The mean of 19 normal EDTA plasma was 663 pg/ml (range: 206 to 2307 pg/ml).

Culture Stimulation

Human PBMCs, whole blood or skin fibroblasts (5) can be stimulated for 24 or 48 hours with PMA (phorbol 12-myristate 13-acetate). This procedure results in the production of up to 720 pg/ml of Hu HGF in undiluted whole blood. Human dermal fibroblasts can be stimulated with LTA (*Staphylococcus aureus* lipoteichoic acid) and PA (Protein-A) to induce HGF (6).

Limitations of the Procedure

Do not extrapolate the standard curve beyond the top standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute all samples above the top standard point with *Standard Diluent Buffer*; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Hu HGF in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

Appendix

Troubleshooting Guide

Elevated background	*Cause:* Insufficient washing and/or draining of wells after washing. Solution containing either biotin or SAV-HRP can elevate the background if residual is left in the well. *Solution:* Wash according to the protocol. Verify the function of automated plate washer. At the end of each washing step, invert plate on absorbent tissue on countertop and allow to completely drain and tap forcefully if necessary to remove residual fluid. *Cause:* Contamination of substrate solution with metal ions or oxidizing reagents. *Solution:* Use distilled/deionized water for dilution of wash buffer and use plastic equipment. DO NOT COVER plate with foil. *Cause:* Contamination of pipette, dispensing reservoir or substrate solution with SAV-HRP conjugate. *Solution:* Do not use chromogen that appears blue prior to dispensing onto the plate. Obtain new vial of chromogen. *Cause:* Incubation time is too long or incubation temperature is too high. *Solution:* Reduce incubation time and/or temperature.
Elevated sample/ standard ODs	*Cause:* Incorrect dilution of standard stock solution; intermediary dilutions not followed correctly. *Solution*: Follow the protocol instructions regarding the dilution of the standard. *Cause:* Incorrect dilution of the SAV-HRP conjugate. *Solution:* Warm solution of SAV-HRP concentrate to room temperature, draw up slowly and wipe tip with kim-wipe to remove excess. Dilute ONLY in SAV diluent provided. *Cause*: Incubation times extended. *Solution:* Follow incubation times outlined in protocol. *Cause:* Incubations carried out at 37°C when RT is dictated. *Solution:* Perform incubations at RT (= 25 ± 2°C) when instructed in the protocol.
Poor standard curve	*Cause:* Improper preparation of standard stock solution. *Solution:* Dilute lyophilized standard as directed by the vial label only with the standard diluent buffer or in a diluent that most closely matches the matrix of your sample. *Cause:* Reagents (lyophilized standard, standard diluent buffer, etc.) from different kits, either different cytokine or different lot number, were substituted. *Solution:* NEVER substitute any components from another kit. *Cause*: Errors in pipetting the standard or subsequent steps. *Solution*: Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device.

Weak/no color develops	*Cause:* Reagents not at RT (25 ± 2°C) at start of assay. *Solution:* Allow ALL reagents to warm to RT prior to commencing assay. *Cause:* Incorrect storage of components, e.g., not stored at 2 to 8°C. *Solution:* Store all components exactly as directed in protocol and on labels. *Cause:* Working SAV-HRP solution made up longer than 15 minutes before use in assay. *Solution:* Use the diluted SAV-HRP within 15 minutes of dilution. *Cause:* TMB solution lost activity. *Solution 1:* The TMB solution should be clear before it is dispensed into the wells of the microtiter plate. An intense aqua blue color indicates that the product is contaminated. Please contact Technical Support if this problem is noted. To avoid contamination, we recommend that the quantity required for an assay be dispensed into a disposable trough for pipetting. Any TMB solution left in the trough should be discarded. *Solution 2:* Avoid contact of the TMB solution with items containing metal ions. *Cause:* Attempt to measure analyte in a matrix for which the ELISA assay has not been optimized. *Solution:* Please contact Technical Support for advice when using nonvalidated sample types. *Cause:* Wells have been scratched with pipette tip or washing tips. *Solution:* Use caution when dispensing and aspirating into and out of microwells.
Poor Precision	*Cause:* Errors in pipetting the standards, samples or subsequent steps. *Solution:* Always dispense into wells quickly and in the same order. Do not touch the pipette tip on the individual microwells when dispensing. Use calibrated pipettes and the appropriate tips for that device. Check for any leaks in the pipette tip. *Cause:* Repetitive use of tips for several samples or different reagents. *Solution:* Use fresh tips for each sample or reagent transfer. *Cause:* Wells have been scratched with pipette tip or washing tips. *Solution:* Use caution when dispensing and aspirating into and out of microwells.

Technical Support

Contact Us

For more troubleshooting tips, information, or assistance, please call, email, or go online to www.invitrogen.com/ELISA.

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5. Gohda, E., et al. (1992) Phorbol ester-induced secretion of human hepatocyte growth factor by human skin fibroblasts and its inhibition by dexamethasone. *FEBS Lett.* 301(1):107-110.
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Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

Human HGF Assay Summary

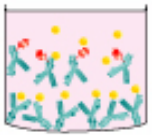
Add 150 µL
Incubation Buffer into each well

Add 50 µL
Standard / Control / Sample

Incubate for 3 hours at RT

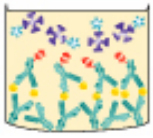
aspirate and wash 4x

Incubate 100 µL of Biotin Conjugate
for 1 hour at RT



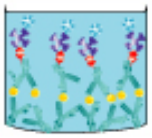
aspirate and wash 4x

Incubate 100 µL of Streptavidin-HRP
Working Solution for 30 minutes at RT

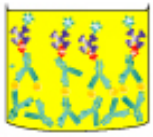


aspirate and wash 4x

Incubate 100 µL of Stabilized chromogen
for 30 minutes at RT



Add 100 µL Stop Solution
and read plate at 450 nm



Total time: 5 hours