

# Quantikine<sup>®</sup>

## Human HGF Immunoassay

Catalog Number DHG00

SHG00

PDHG00

**For the quantitative determination of human hepatocyte growth factor (HGF) concentrations in cell culture supernates, serum, and plasma.**

*This package insert must be read in its entirety before using this product.*

**FOR RESEARCH USE ONLY.  
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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## INTRODUCTION

Hepatocyte growth factor (HGF) is a pleiotropic growth factor originally isolated from rat platelets (1, 2). This factor has also been called scatter factor, hepatopoietin A (hepatopoietin B is likely a fragment of A) and mammary growth factor (3). Mature HGF is an 82 kDa, 674 amino acid residue heterodimeric glycoprotein that is part of a small family of factors that also includes an HGF-like factor known as macrophage stimulating protein (MSP). These factors lack significant homology with other known growth factors (4 - 7). HGF has marked and varied effects on hepatocytes and other epithelial cells, as well as on endothelial, mesenchymal and hematopoietic cell types, including mitogenic, morphogenic, and motogenic activities (6, 8, 9). HGF demonstrates marked species cross-reactivity (10). For reviews on HGF, see references 6, 8, 9, 11 - 13.

HGF is synthesized as a single chain, 728 amino acid residue pre-pro-peptide with a 29 amino acid signal sequence and a 25 amino acid pro-sequence. Mature HGF is formed by the activity of a unique serine protease that extracellularly cleaves a pro-sequence Arg-Val linkage, an activity that itself is activated by another protease in response to tissue damage (6, 14 - 19). The resulting molecule consists of a disulfide-linked 69 kDa  $\alpha$ -chain 440 amino acids in length with a predicted molecular weight of 50,800 and a 34 kDa  $\beta$ -chain 234 amino acids in length with a predicted molecular weight of 26,000. Glycosylation presumably accounts for the differences between the predicted and observed molecular sizes of the two subunits (15). Each subunit has two potential N-linked glycosylation sites and an O-linked carbohydrate has been detected on the  $\alpha$ -chain (15, 20).

The biological actions of HGF are mediated by a high affinity membrane-bound receptor identified as the *c-met* proto-oncogene product (9, 21 - 23). The *c-met* proto-oncogene is a 1408 amino acid glycoprotein. This receptor is part of a family that also includes the products of the *Ron* (the receptor for MSP) and *Sea* genes (6, 9). These receptors exhibit no significant homology to any other known growth factor receptors. The most common (190 kDa) isoform of the receptor undergoes considerable post-translational modification of the extracellular region, including glycosylation on one or more of eleven potential N-linked extracellular glycosylation sites, followed by proteolytic cleavage into disulfide-linked 50 kDa  $\alpha$ - and 145 kDa  $\beta$ - chains (25). Glycosylation of these sites is considered a prerequisite for subsequent proteolytic cleavage. The resultant heterodimer consists of an N-terminal 283 amino acid  $\alpha$ -chain that is strictly extracellular and a C-terminal 1101 amino acid  $\beta$ -chain that contains the remaining extracellular, transmembrane and cytoplasmic domains (25, 26). At least two additional isoforms of the receptor have been reported. Both are possibly functional based on their ability to be phosphorylated in an *in vitro* kinase assay and both are suggested to arise via alternative splicing events. Although the first isoform gives rise to both  $\alpha$ - and  $\beta$ -chains, an 18 amino acid segment is missing from the extracellular portion of the  $\beta$ -subunit. The second isoform, although expressed at the cell surface, never undergoes cleavage with heterodimer formation (27). The significance of these variant receptor forms is unknown.

The presence of HGF mRNA or HGF has been demonstrated in a variety of tissue types. The highest levels of HGF mRNA are found in adult lung, liver, skin and spleen. Detectable mRNA levels have also been found in blood cells, brain, bone marrow, kidney, and the placenta (6). Cells shown to express HGF mRNA include megakaryocytes of various tissues, monocytes, platelets, fibroblasts, smooth muscle cells, mast cells, and endothelial cells, but not epithelial cells (6). Expression of the HGF receptor, on the other hand, is found mainly in epithelial cells, suggesting that HGF acts in a paracrine fashion to mediate interactions between epithelial and stromal cells during development and in normal tissue maintenance (6). Elevated levels of HGF reportedly have been found in the serum of individuals with a variety of liver disorders (28, 29) and with various types of leukemias or lymphomas (30).

HGF is pleiotropic in its activities which include mitogenesis, motogenesis, morphogenesis, and growth inhibition. For various types of epithelial and endothelial cells, HGF is a potent mitogen. In particular, HGF is known to stimulate the growth and DNA synthesis of normal epidermal melanocytes, keratinocytes, renal tubular epithelium, gastric epithelium, biliary epithelium, vascular endothelium and normal liver hepatocytes (31 - 38). HGF also stimulates proliferation and proteoglycan synthesis for some mesenchymal cells, such as chondrocytes (39). It has been indicated that HGF can also stimulate the proliferation and differentiation of hematopoietic progenitor cells (40). HGF synergizes with erythropoietin to induce the formation of colonies of the erythroid lineage (BFU-E) *in vitro*. In the presence of erythropoietin plus stem cell factor, HGF stimulates the *in vitro* formation of multipotent (CFU-GEMM) colonies. The ability of HGF to promote hepatocyte proliferation is of particular interest because any hepatocyte mitogen has potential clinical application in reversing compromised hepatic function *in vivo* (37, 39 - 44). In addition to a normal role in endothelial cell maintenance, high levels of HGF are now suspected to be responsible for the initial development of Kaposi's sarcoma tumor cells, a process facilitated at a later stage by other cytokines (45).

HGF has also demonstrated motogenic activity towards epithelial, endothelial, and mesenchymal cells. Normal human keratinocytes, Madin-Darby canine kidney (MDCK) epithelial cells, bovine pulmonary artery endothelial cells, human omental microvascular endothelium, and chondrocytes all show marked migration *in vitro* in response to HGF (32, 37, 46). HGF also induces MDCK cells to form branching epithelial tubules in collagen matrix (47). Activities such as these are suggested to complement mitogenesis in the repair and/or regeneration of damaged tissues (42). Less desirably, HGF has been shown to promote the scattering and migration of human MKN-74 gastric adenocarcinoma cells, suggesting a role as a promoter of metastasis (48).

In contrast to its stimulatory activities, HGF also demonstrates inhibitory activity. In select human small (or oat) cell lung carcinoma cell lines, HGF has been noted to inhibit their *in vitro* growth rate by approximately half (49). In addition, HGF has been shown to strongly inhibit the growth of HepG2 hepatocellular carcinoma (HCC) cells, B6/F1 melanoma cells, and KB squamous carcinoma cells in culture via a cytostatic rather than cytolytic mechanism (50).

The Quantikine Human HGF Immunoassay is a 4.25 - 4.5 hour solid phase ELISA that is designed to measure HGF levels in cell culture supernates, serum, and plasma. It contains Sf 21-expressed recombinant human pro-HGF and antibodies raised against the recombinant factor, and has been shown to quantitate recombinant human HGF accurately. Results obtained using natural HGF showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that the Quantikine kit can be used to determine relative mass values for natural HGF.

## **PRINCIPLE OF THE ASSAY**

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for HGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any HGF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for HGF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of HGF bound in the initial step. The color development is stopped and the intensity of the color is measured.

## LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

## MATERIALS PROVIDED

Description	Part #	Cat. # DHG00	Cat. # SHG00
<b>HGF Microplate</b> - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against HGF.	890235	1 plate	6 plates
<b>HGF Conjugate</b> - 21 mL/vial of polyclonal antibody against HGF conjugated to horseradish peroxidase with preservatives.	890236	1 vial	6 vials
<b>HGF Standard</b> - 80 ng/vial of recombinant human pro-HGF in a buffered protein base with preservatives; lyophilized.	890237	1 vial	6 vials
<b>Assay Diluent RD1W</b> - 11 mL/vial of a buffered protein base with preservatives.	895117	2 vials	12 vials
<b>Calibrator Diluent RD5P Concentrate (5X)</b> - 21 mL/vial of a concentrated buffered protein base with preservatives. <i>For cell culture supernate samples.</i>	895151	1 vial	6 vials
<b>Calibrator Diluent RD6X</b> - 21 mL/vial of animal serum with preservatives. <i>For serum/plasma samples.</i>	895152	1 vial	6 vials
<b>Wash Buffer Concentrate</b> - 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives.	895003	1 vial	6 vials
<b>Color Reagent A</b> - 12.5 mL/vial of stabilized hydrogen peroxide.	895000	1 vial	6 vials
<b>Color Reagent B</b> - 12.5 mL/vial of stabilized chromogen (tetramethylbenzidine).	895001	1 vial	6 vials
<b>Stop Solution</b> - 6 mL/vial of 2 N sulfuric acid.	895032	1 vial	6 vials
<b>Plate Covers</b> - Adhesive strips.	—	4 strips	24 strips

DHG00 contains sufficient materials to run an ELISA on one 96 well plate.

SHG00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDGH00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

## STORAGE

<b>Unopened Kit</b>	Store at 2 - 8° C. Do not use past kit expiration date.	
<b>Opened/ Reconstituted Reagents</b>	Diluted Wash Buffer	May be stored for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Calibrator Diluent RD5P (1X)	
	Calibrator Diluent RD6X	
	Assay Diluent RD1W	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	Aliquot and store for up to 1 month at ≤ -20° C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up 1 month at 2 - 8° C.*

\*Provided this is within the expiration date of the kit.

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Human HGF Controls (optional; available from R&D Systems).
- **Polypropylene** test tubes for serial dilution.

## PRECAUTIONS

HGF is detectable in saliva. Take precautionary measures to prevent contamination of the kit reagents while running the assay.

The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

Calibrator Diluent RD6X contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

## SAMPLE COLLECTION AND STORAGE

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

**Note:** *Heparinized plasma is not recommended for use in this assay.*

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

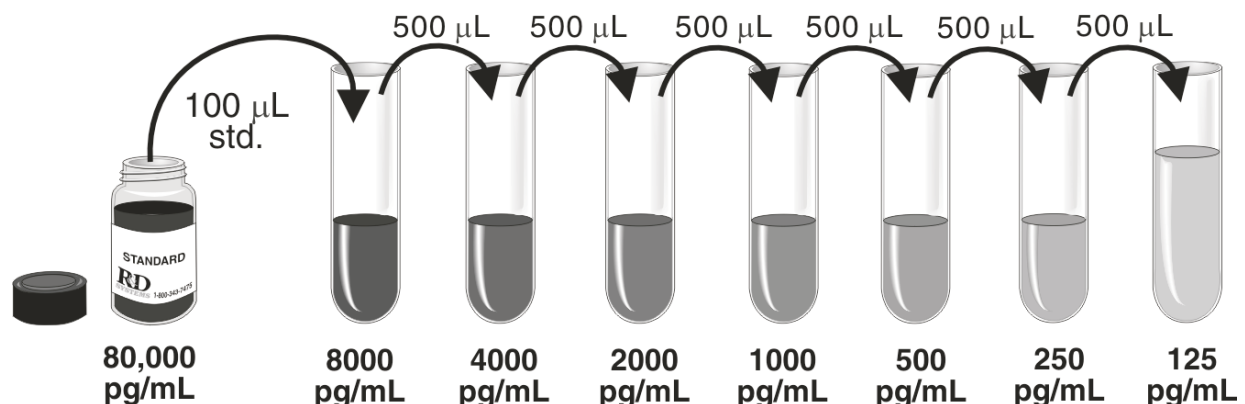
**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200  $\mu$ L of the resultant mixture is required per well.

**Calibrator Diluent RD5P (1X)** - Dilute 20 mL of Calibrator Diluent RD5P Concentrate into deionized or distilled water to yield 100 mL of Calibrator Diluent RD5P (1X).

**HGF Standard** - Reconstitute the HGF Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 80,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

**Use polypropylene tubes.** Pipette 900  $\mu$ L of Calibrator Diluent RD5P (1X) (*for cell culture supernate samples*) or Calibrator Diluent RD6X (*for serum/plasma samples*) into the 8000 pg/mL tube. Pipette 500  $\mu$ L of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 8000 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 pg/mL).



## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate.**

1. Prepare all reagents, samples, and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 150  $\mu\text{L}$  of Assay Diluent RD1W per well.
4. Add 50  $\mu\text{L}$  of Standard, control, or sample per well. Ensure reagent addition is uninterrupted and completed **within 15 minutes**. Mix by gently tapping the plate. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400  $\mu\text{L}$ ) using a squirt bottle, manifold dispenser, 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.
6. Add 200  $\mu\text{L}$  of HGF Conjugate to each well. Cover with a new adhesive strip.  
**For Serum/Plasma Samples:** Incubate for 2 hours at room temperature.  
**For Cell Culture Supernate Samples:** Incubate for 1.75 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200  $\mu\text{L}$  of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50  $\mu\text{L}$  of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

## ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and standards as instructed.



2. Add 150  $\mu\text{L}$  Assay Diluent RD1W to each well.



3. Add 50  $\mu\text{L}$  Standard, control, or sample to each well within 15 minutes. Mix.  
Incubate 2 hours at RT.

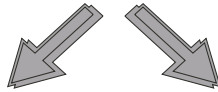


4. Aspirate and wash 4 times.



5. Add 200  $\mu\text{L}$  Conjugate to each well.

**Serum/Plasma  
Samples**



**Cell Culture Supernate  
Samples**

Incubate 2 hours at RT.



Incubate 1.75 hours at RT.

6. Aspirate and wash 4 times.



7. Add 200  $\mu\text{L}$  Substrate Solution to each well.  
Incubate 30 minutes at RT.

**Protect from light.**



8. Add 50  $\mu\text{L}$  Stop Solution to each well.  
Read at 450 nm within 30 minutes.  
 $\lambda$  correction 540 or 570 nm

# CALCULATION OF RESULTS

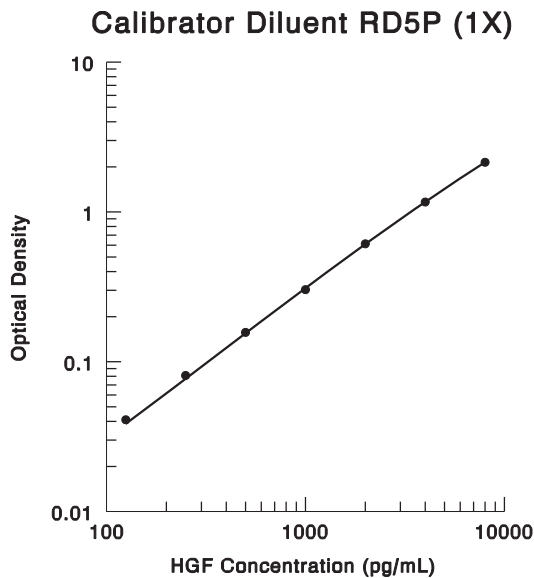
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the HGF concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

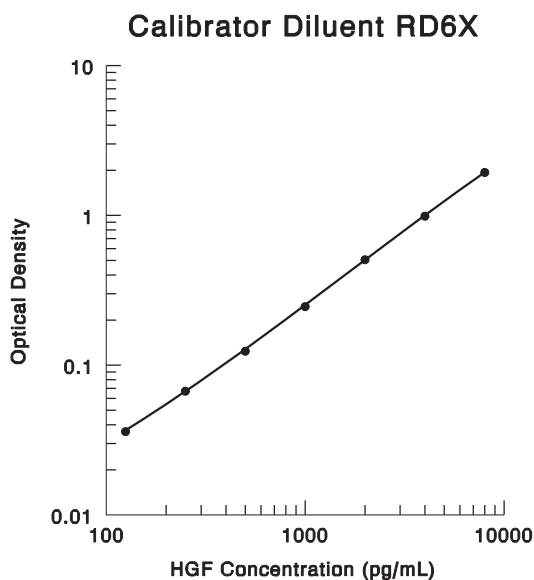
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## TYPICAL DATA

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



(pg/mL)	O.D.	Average	Corrected
0	0.046 0.048 0.089	0.047	—
125	0.087 0.128	0.088	0.041
250	0.127 0.206	0.128	0.081
500	0.203 0.366	0.204	0.157
1000	0.333 0.649	0.350	0.303
2000	0.672 1.199	0.660	0.613
4000	1.226 2.216	1.212	1.165
8000	2.167	2.192	2.145



(pg/mL)	O.D.	Average	Corrected
0	0.055 0.057 0.091	0.056	—
125	0.094 0.125	0.092	0.036
250	0.121 0.182	0.123	0.067
500	0.178 0.311	0.180	0.124
1000	0.294 0.580	0.302	0.246
2000	0.543 1.067	0.562	0.506
4000	1.020 2.074	1.044	0.988
8000	1.908	1.991	1.935

## TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

## PRECISION

### Intra-assay Precision (Precision within an assay)

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

### Inter-assay Precision (Precision between assays)

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

### Serum/Plasma Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	339	999	3759	363	967	3790
Standard deviation	23.7	56.2	154	25.8	81.2	205
CV (%)	7.0	5.6	4.1	7.1	8.4	5.4

### Cell Culture Supernate Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	425	991	3869	409	1058	4058
Standard deviation	30.2	43.2	234	29.4	75.1	264
CV (%)	7.1	4.4	6.0	7.2	7.1	6.5

## RECOVERY

The recovery of HGF spiked to three different levels in five samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media	92	86 - 101%
Serum	99	91 - 108%
EDTA plasma	100	85 - 111%
Citrate plasma	105	99 - 114%

## LINEARITY

To assess the linearity of the assay, five samples of each sample type were spiked with high concentrations of HGF and diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media	Serum	EDTA plasma	Citrate plasma
1:2	Average % of Expected	104	100	100	102
	Range (%)	95-107	98-101	97-104	98-106
1:4	Average % of Expected	104	97	98	103
	Range (%)	98-107	96-99	93-107	97-111
1:8	Average % of Expected	106	96	97	101
	Range (%)	102-108	93-100	89-109	97-107
1:16	Average % of Expected	104	96	98	102
	Range (%)	94-109	92-106	86-110	93-113

## SENSITIVITY

The minimum detectable dose of HGF is typically less than 40 pg/mL.

The minimum detectable dose was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

## CALIBRATION

This immunoassay is calibrated against a highly purified *Sf* 21-expressed recombinant human pro-HGF produced at R&D Systems.

To convert sample values obtained with the Quantikine Human HGF kit to approximate NIBSC 95/556 International Units, use the equation below.

NIBSC (95/556) approximate value (IU/mL) = 0.0003 x Quantikine Human HGF value (pg/mL)

## SAMPLE VALUES

**Serum/Plasma** - Samples drawn from apparently healthy volunteers were evaluated for the presence of HGF in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)
Serum (n=71)	1257	671 - 1992
EDTA plasma (n=71)	787	469 - 1113
Citrate plasma (n=71)	431	251 - 742

**Cell Culture Supernates** - Human peripheral blood mononuclear cells ( $5 \times 10^6$  cells/mL) were cultured in RPMI supplemented with 5% fetal calf serum, 50  $\mu$ M  $\beta$ -mercaptoethanol, 2 mM L-glutamine, 100 units/mL penicillin, and 100  $\mu$ g/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10  $\mu$ g/mL PHA for 1 and 5 days.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	548
Stimulated	152	145

ND = Non-detectable

## SPECIFICITY

This assay recognizes both natural and recombinant human HGF. The factors listed below were prepared at 50 ng/mL in each Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhHGF control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

ANG	IL-1ra
AR	IL-1 sRI
CNTF	IL-1 sRII
$\beta$ -ECGF	IL-2
EGF	IL-2 sR $\alpha$
Epo	IL-3
FGF acidic	IL-3 sR $\alpha$
FGF basic	IL-4
FGF-4	IL-4 sR $\alpha$
FGF-5	IL-5
FGF-6	IL-5 sR $\alpha$
G-CSF	IL-5 sR $\beta$
GM-CSF	IL-6
sgp130	IL-6 sR
GRO $\alpha$	IL-7
GRO $\beta$	IL-8
GRO $\gamma$	IL-9
HB-EGF	IL-10
HGF R	IL-11
IFN- $\gamma$	IL-12
IGF-I	IL-13
IGF-II	KGF
IL-1 $\alpha$	LAP (TGF- $\beta$ 1)
IL-1 $\beta$	LIF

### Recombinant mouse:

GM-CSF
HGF
IL-1 $\alpha$
IL-1 $\beta$
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-13
LIF
MIP-1 $\alpha$
MIP-1 $\beta$
SCF
TNF- $\alpha$

### Recombinant amphibian:

TGF- $\beta$ 5
----------------

### Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF
human TGF- $\beta$ 1
porcine TGF- $\beta$ 1
porcine TGF- $\beta$ 2

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# PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

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