# Quantikine® ELISA

## Human HGF Immunoassay

Catalog Number DHG00B
SHG00B
PDHG00B

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.

# TABLE OF CONTENTS

## SECTION PAGE




**Manufactured and Distributed by:**

**USA** R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
**TEL:** 800 343 7475 612 379 2956
**FAX:** 612 656 4400
**E-MAIL:** info@bio-techne.com

**Distributed by:**

**Europe | Middle East | Africa** Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
**TEL:** +44 (0)1235 529449
**FAX:** +44 (0)1235 533420
**E-MAIL:** info.emea@bio-techne.com

**China** Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
**TEL:** +86 (21) 52380373 (400) 821-3475
**FAX:** +86 (21) 52371001
**E-MAIL:** info.cn@bio-techne.com

# INTRODUCTION

Hepatocyte Growth Factor (HGF), also known as scatter factor, hepatopoietin A, and mammary growth factor, is a pleiotropic glycoprotein that regulates the growth and migration of diverse cell types. It is structurally similar to the S1 peptidase Plasminogen. HGF contains an N-terminal PAN/APPLE-like domain, four Kringle domains, and a catalytically inactive serine proteinase-like domain (1, 2). Alternative splicing generates human HGF isoforms that lack the proteinase-like domain and different numbers of the Kringle domains. HGF is secreted as an inactive single chain propeptide that can circulate as a soluble molecule or associate with the extracellular matrix (3, 4). At sites of tissue damage, the propeptide is cleaved after the fourth Kringle domain by serine proteases including HGF Activator and uPA (4-8). The resulting bioactive HGF consists of a disulfide-linked heterodimer of a 60 kDa N-terminal alpha chain and a 30 kDa C-terminal beta chain (4, 5, 9). The serum levels of HGF are elevated in a wide range of pathologies including liver damage (10, 11), acute kidney failure (12), myocardial infarction (13), type 1 diabetes (14), obesity (15), and cancer (16-23), as well as in the synovial fluid of rheumatoid arthritis patients (24). Human HGF shares 91-94% amino acid sequence identity with bovine, canine, feline, mouse, and rat HGF. HGF demonstrates marked species cross-reactivity (25).

HGF exerts its biological activity through the widely expressed receptor tyrosine kinase, HGF R/c-MET (26, 27). This receptor undergoes N-linked glycosylation followed by proteolytic cleavage into 50 kDa N-terminal alpha and 145 kDa C-terminal beta chains (28). The strictly extracellular alpha chain remains disulfide-linked to the beta chain which contains the remaining extracellular, transmembrane, and cytoplasmic domains (26, 27). HGF also binds heparan sulfate proteoglycans, and these interactions enhance the ability of HGF to bind and activate HGF R (29, 30). In the absence of ligand, HGF R forms noncovalent complexes with a variety of membrane proteins including CD44v6, CD151, EGF R, Fas, Integrin α6/β4, Plexins B1, B2, B3, and MSP R/Ron (31-38). Ligation of one complex component can trigger activation of the other, followed by cooperative signaling effects (31-38). Formation of some of these heteromeric complexes is a requirement for epithelial cell morphogenesis and tumor cell invasion (34-36). Overexpression and the production of alternate forms of HGF R are implicated in the development of many human cancers (39).

HGF is expressed by fibroblasts, adipocytes, smooth muscle cells, and endothelial cells (1). Expression of HGF R, on the other hand, is found mainly on epithelial cells, suggesting that HGF acts in a paracrine fashion to mediate interactions between stromal and epithelial cells (40). HGF induces the proliferation and migration of epithelial cells as well as multiple other cell types including hepatocytes, chondrocytes, keratinocytes, melanocytes, and endothelial cells (1). It is mitogenic toward most tumor cells but can conversely inhibit their proliferation in some cases (39, 41, 42). During organogenesis, tissue repair, and angiogenesis, HGF promotes epithelial/endothelial morphogenesis by inducing cell scattering and branching tubulogenesis (1, 25, 43, 44). The ability of HGF to regulate angiogenesis and the motility of epithelial cells underlie its importance in the development of solid tumors (39). In addition to its morphogenetic effects, HGF induces a range of responses in diverse tissues (1). It supports the survival, proliferation, and insulin productivity of pancreatic islet cells (45). It functions as a neurotrophic factor during development and in the response to injury (46, 47). It also suppresses inflammation by inducing dendritic cell tolerization, Treg induction, and Th2 bias while inhibiting T cell activation, IL-17 expression, and inflammatory cell infiltration (48-50).

The Quantikine® Human HGF Immunoassay is a 4.0 hour solid phase ELISA that is designed to measure human HGF levels in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human HGF and antibodies raised against the recombinant factor. It has been shown to quantitate recombinant human HGF. Results obtained using natural human HGF showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human HGF.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human 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 monoyclonal antibody specific for human 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.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

## 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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.
- 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.

## MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

| PART | PART # | CATALOG # DHG00B | CATALOG # SHG00B | DESCRIPTION | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|---|---|---|
| **Human HGF Microplate** | 898976 | 1 plate | 6 plates | 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human HGF. | Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.* |
| **Human HGF Standard** | 898978 | 2 vials | 12 vials | Recombinant human HGF in a buffered protein base with preservatives; lyophilized. *Refer to vial label for reconstitution volume.* | Use a fresh standard for each assay. Discard after use. |
| **Human HGF Conjugate** | 898977 | 1 vial | 6 vials | 21 mL/vial of a monoclonal antibody specific for human HGF conjugated to biotin with preservatives. | May be stored for up to 1 month at 2-8 °C.* |
| **Streptavidin-HRP 1** | 898926 | 1 vial | 6 vials | 21 mL of a solution with preservatives. | |
| **Assay Diluent RD1-38** | 895301 | 1 vial | 6 vials | 11 mL/vial of a buffered protein base with preservatives. | |
| **Calibrator Diluent RD5P** | 895151 | 1 vial | 6 vials | 21 mL/vial of a concentrated buffered protein base with preservatives. *Use diluted 1:5 in this assay.* | |
| **Wash Buffer Concentrate** | 895003 | 2 vials | 12 vials | 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives. *May turn yellow over time.* | |
| **Color Reagent A** | 895000 | 1 vial | 6 vials | 12 mL/vial of stabilized hydrogen peroxide. | |
| **Color Reagent B** | 895001 | 1 vial | 6 vials | 12 mL/vial of stabilized chromogen (tetramethylbenzidine). | |
| **Stop Solution** | 895032 | 1 vial | 6 vials | 6 mL/vial of 2 N sulfuric acid. | |
| **Plate Sealers** | N/A | 4 strips | 24 strips | Adhesive strips. | |

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

DHG00B contains sufficient materials to run an ELISA on one 96 well plate.
SHG00B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDHG00B). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the Pharmpak Contents section for specific vial counts.

## PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL, and not in the glass vials described in the package insert. **Note:** *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

| PART | PART # | QUANTITY |
|---|---|---|
| **Human HGF Microplate** | 898976 | 50 plates |
| **Human HGF Standard** | 898978 | 25 vials |
| **Human HGF Conjugate** | 898977 | 50 vials |
| **Streptavidin-HRP 1** | 898926 | 50 vials |
| **Assay Diluent RD1-38** | 895301 | 50 vials |
| **Calibrator Diluent RD5P** | 895151 | 10 vials |
| **Wash Buffer Concentrate** | 895126 | 12 bottles |
| **Color Reagent A** | 895000 | 50 vials |
| **Color Reagent B** | 895001 | 50 vials |
| **Stop Solution** | 895032 | 50 vials |
| **Plate Sealers** | N/A | 200 sheets |
| **Package Inserts** | 753425 | 2 booklets |

## 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.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards.
- Human HGF Controls (R&D Systems, Catalog # QC247).

## PRECAUTIONS

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

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

## SAMPLE COLLECTION & STORAGE

**The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.**

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA, heparin, 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 ≤ - 20 °C. Avoid repeated freeze-thaw cycles.

## REAGENT PREPARATION

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

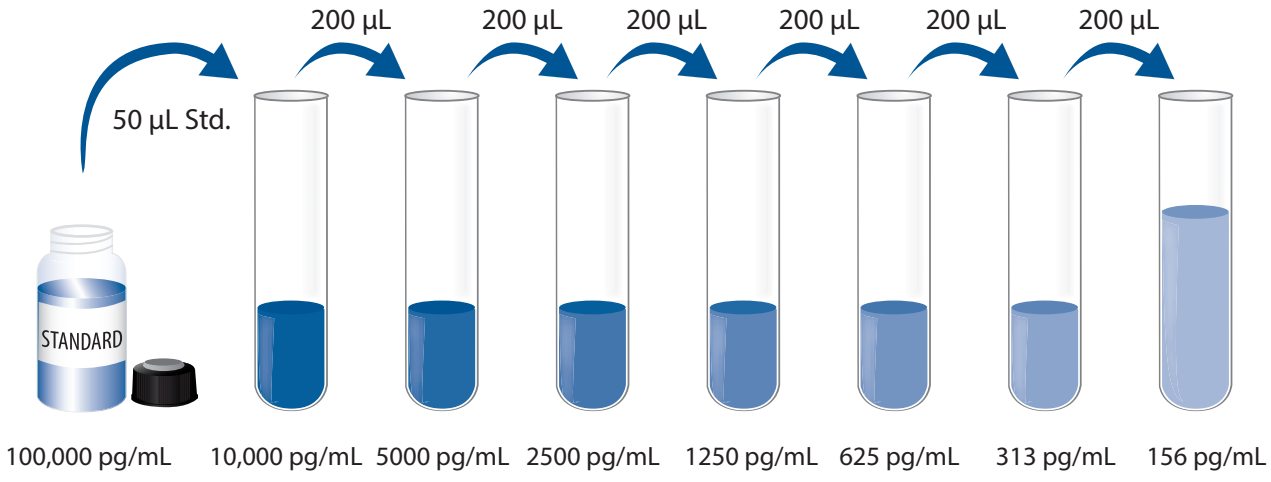
**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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

**Calibrator Diluent RD5P (diluted 1:5)** - Add 10 mL of Calibrator Diluent RD5P to 40 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5P (diluted 1:5).

**Human HGF Standard** - **Refer to the vial label for reconstitution volume.** Reconstitute the Human HGF Standard with deionized or distilled water. This reconstitution produces a stock solution of 100,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

**Use polypropylene tubes.** Pipette 450 µL of Calibrator Diluent RD5P (diluted 1:5) into the 10,000 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10,000 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) 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 standards, controls, and samples be assayed in duplicate.**

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

1. Prepare all reagents 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 100 µL of Assay Diluent RD1-38 to each well.
4. Add 50 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for **2 hours** at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. 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 µL) 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 µL of Human HGF Conjugate to each well. Cover with a new adhesive strip. Incubate for **1 hour** at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Streptavidin-HRP 1 to each well. Cover with a new adhesive strip. Incubate for **30 minutes** at room temperature on the shaker.
9. Repeat the aspiration/wash as in step 5.
10. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
11. Add 50 µL 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.
12. 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.

## CALCULATION OF RESULTS

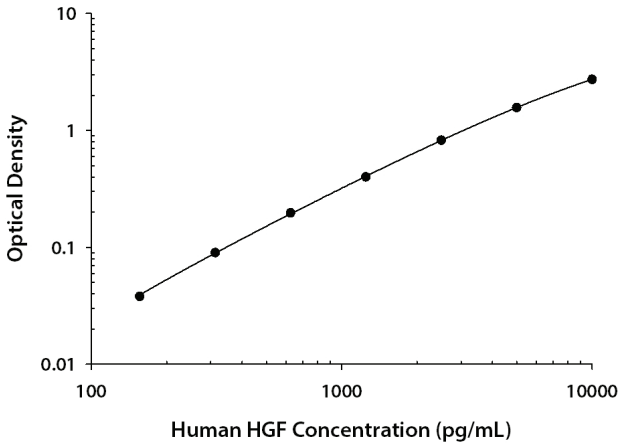
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human 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

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




| (pg/mL) | O.D. | Average | Corrected |
|---|---|---|---|
| 0 | 0.003<br>0.008 | 0.006 | — |
| 156 | 0.043<br>0.044 | 0.044 | 0.038 |
| 313 | 0.094<br>0.097 | 0.096 | 0.090 |
| 625 | 0.200<br>0.206 | 0.203 | 0.197 |
| 1250 | 0.403<br>0.409 | 0.406 | 0.400 |
| 2500 | 0.826<br>0.836 | 0.831 | 0.825 |
| 5000 | 1.569<br>1.579 | 1.574 | 1.568 |
| 10,000 | 2.734<br>2.743 | 2.739 | 2.733 |

## 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 twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

| | Intra-Assay Precision | | | Inter-Assay Precision | | |
|---|---|---|---|---|---|---|
| **Sample** | **1** | **2** | **3** | **1** | **2** | **3** |
| **n** | 20 | 20 | 20 | 20 | 20 | 20 |
| **Mean (pg/mL)** | 1296 | 2626 | 5080 | 1414 | 2755 | 5410 |
| **Standard deviation** | 30.2 | 75.8 | 147 | 112 | 176 | 212 |
| **CV (%)** | 2.3 | 2.9 | 2.9 | 7.9 | 6.4 | 3.9 |

## RECOVERY

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

| Sample Type | Average % Recovery | Range |
|---|---|---|
| Cell culture media (n=4) | 107 | 101-118% |
| Serum (n=4) | 104 | 94-115% |
| EDTA plasma (n=4) | 104 | 95-114% |
| Heparin plasma (n=4) | 113 | 96-125% |
| Citrate plasma (n=4) | 102 | 91-115% |

## SENSITIVITY

Twenty-three assays were evaluated and the minimum detectable dose (MDD) of human HGF ranged from 2.71-24.1 pg/mL. The mean MDD was 9.78 pg/mL.

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

## LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human HGF were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

| | | Cell culture supernates* (n=4) | Serum (n=4) | EDTA plasma (n=4) | Heparin plasma (n=4) | Citrate plasma (n=4) |
|---|---|---|---|---|---|---|
| 1:2 | Average % of Expected | 97 | 94 | 97 | 96 | 96 |
| | Range (%) | 92-102 | 91-98 | 90-104 | 89-103 | 89-103 |
| 1:4 | Average % of Expected | 98 | 92 | 95 | 91 | 93 |
| | Range (%) | 92-105 | 87-99 | 90-101 | 86-96 | 88-98 |
| 1:8 | Average % of Expected | 99 | 90 | 92 | 87 | 92 |
| | Range (%) | 92-105 | 86-93 | 86-100 | 81-95 | 85-99 |
| 1:16 | Average % of Expected | 98 | 91 | 91 | 87 | 93 |
| | Range (%) | 95-100 | 86-95 | 87-96 | 82-91 | 87-97 |

*Samples were diluted prior to assay.

## CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human HGF produced at R&D Systems®.

The NIBSC/WHO HGF International Standard 96/556 was evaluated in this kit. The dose response curve of the reference reagent 96/556 parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Human HGF kit to approximate NIBSC/WHO 96/556 Units, use the equation below.

NIBSC/WHO (96/556) approximate value (IU/mL) = 0.0002 x Quantikine® Human HGF value (pg/mL)

The NIBSC/WHO HGF Reference Reagent 96/564 was evaluated in this kit. The dose response curve of the reference reagent 96/564 parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Human HGF kit to approximate NIBSC/WHO 96/564 Units, use the equation below.

NIBSC/WHO (96/564) approximate value (IU/mL) = 0.0005 x Quantikine® Human HGF value (pg/mL)

**Note:** *Based on data generated in May 2018.*

## SAMPLE VALUES

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

| Sample Type | Mean (pg/mL) | Range (pg/mL) | Standard Deviation (pg/mL) |
|---|---|---|---|
| Serum (n=36) | 1440 | 999-3053 | 440 |
| EDTA plasma (n=36) | 780 | 551-1346 | 158 |
| Heparin plasma (n=36) | 821 | 545-1622 | 219 |
| Citrate plasma (n=36) | 644 | 469-1095 | 129 |

**Cell Culture Supernates** - Human peripheral blood mononuclear cells (5 x $10^6$ cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human HGF.

| Condition | Day 1 (pg/mL) | Day 5 (pg/mL) |
|---|---|---|
| Unstimulated | ND | 775 |
| Stimulated | ND | ND |

ND=Non-detectable

U-118 MG human glioblastoma/astrocytoma cells were cultured in DMEM supplemented with 10% fetal bovine serum until confluent. The cells were then washed with PBS before adding serum-free media and cultured for for an additional 24 hours. After the 72 hour incubation, the serum-free media was taken off and centrifuged to remove any cells or debris. An aliquot of the cell culture supernate was removed, assayed for human HGF, and measured 10,079 pg/mL.

Human primary subcutaneous pre-adipocytes were differentiated into adipocytes over a period of 28 days using media containing Subcutaneous Preadipocyte Growth Media, Adipocyte Differentiation Media, and Adipocyte Maintenance Media. On day 28, an aliquot of the media was taken off and centrifuged to remove any cells or debris. An aliquot of the cell culture supernate was removed, assayed for human HGF, and measured 726 pg/mL.

THP-1 human acute monocytic leukemia cells were cultured to a density of ~1 x$10^6$/mL in RPMI 1640 supplemented with 10% fetal bovine serum and 50 µM β-mercaptoethanol. The cells were cultured unstimulated or stimulated with 200 nM PMA for 96 hours prior to conditioned media harvest. An aliquot of the cell culture supernate was taken off and centrifuged to remove any cells or debris. Aliquots of the cell culture supernates were removed, assayed for human HGF, and measured 3435 pg/mL and 953 pg/mL, respectively.

## SPECIFICITY

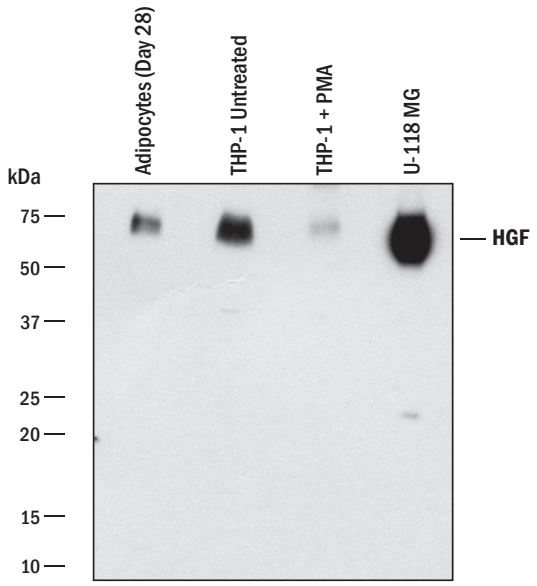
This assay recognizes natural and recombinant human HGF.

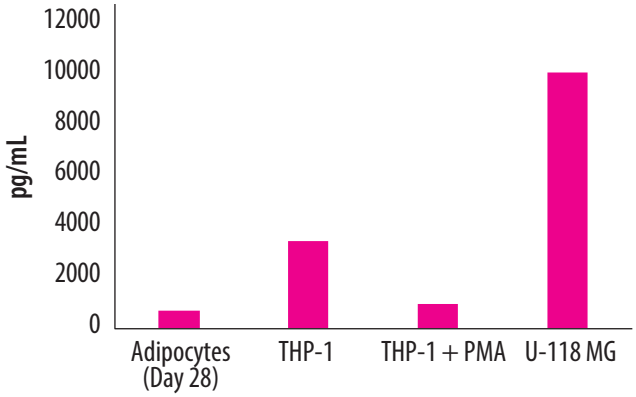
The factors listed below were prepared at 100 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant human HGF control were assayed for interference. No significant cross-reactivity or interference was observed.

**Recombinant human:**
EGF
G-CSF
GM-CSF
HGF R
M-CSF
Met
PDGF-AA
PDGF-AB
PDGF-BB
VEGF

**Other recombinants:**
canine HGF
mouse HGF

**Natural protein:**
human TGF-β1



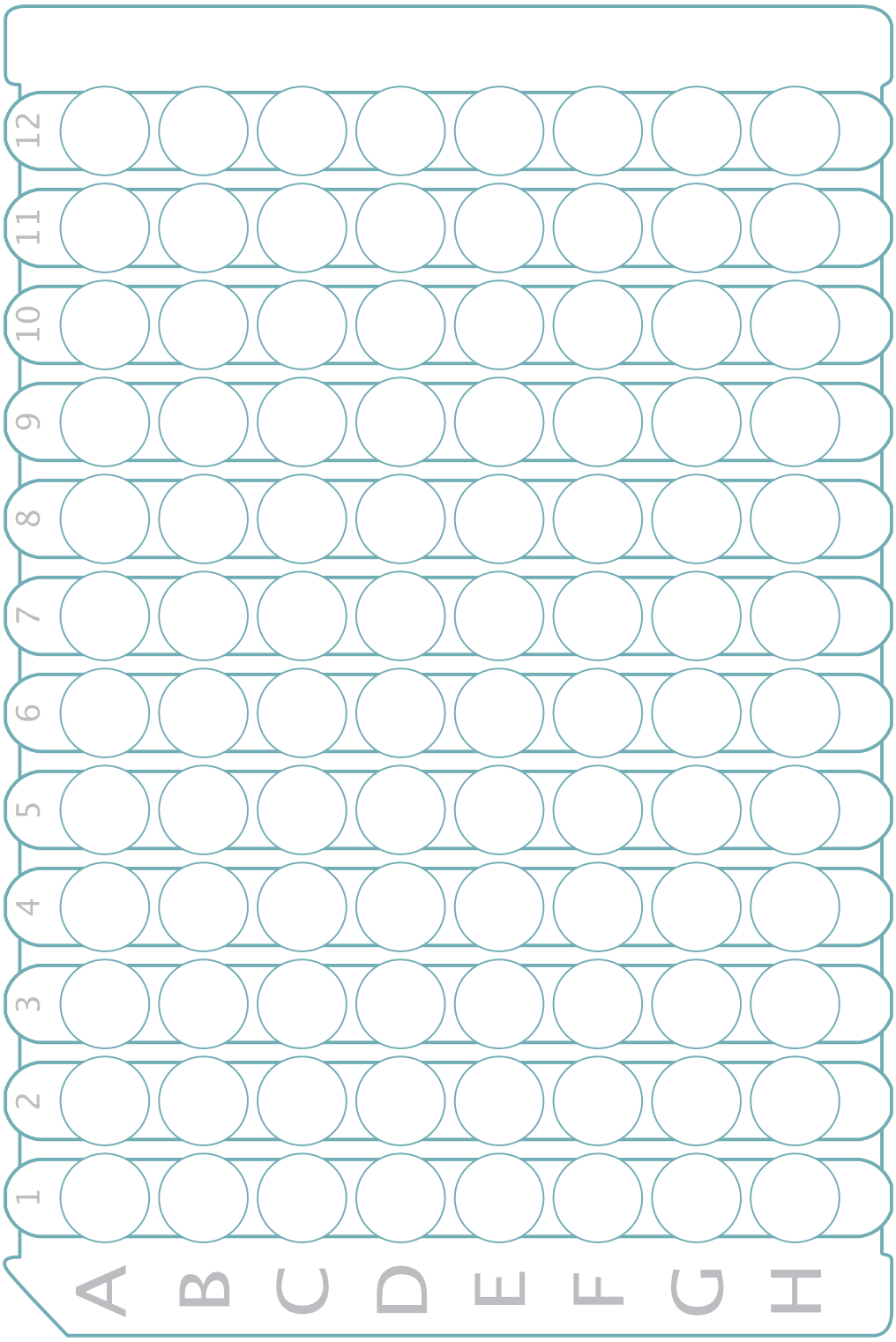

Conditioned media samples were analyzed by Western blot and Quantikine® ELISA. Human preadipocytes were differentiated for 28 days into adipocytes, U-118 MG cells were cultured in serum-free media for 48 hours, and THP-1 cells were left untreated or treated with 200nM PMA for 96 hours prior to collecting cell conditioned media. For Western blot, samples were resolved under non-reducing SDS-PAGE conditions, transferred to a PVDF membrane, and immunoblotted with goat anti-human HGF (R&D Systems®, Catalog # AF-294-NA). The Western Blot shows a direct correlation with ELISA value for these samples.

## REFERENCES


1. Nakamura, T. and S. Mizuno (2010) Proc. Jpn. Acad., Ser. B. **86**:588.
2. Grzelakowska-Sztabert, B. and M. Dudkowska (2011) Growth Factors **4**:105.
3. Sasaki, M. *et al.* (1994) Biochem. Biophys. Res. Commun. **199**:772.
4. Naldini, L. *et al.* (1992) EMBO J. **11**:4825.
5. Naka, D. *et al.* (1992) J. Biol. Chem. **267**:20114.
6. Miyazawa, K. *et al.* (1994) J. Biol. Chem. **269**:8966.
7. Miyazawa, K. *et al.* (1996) J. Biol. Chem. **271**:3615.
8. Sisson, T.H. *et al.* (2009) Blood **114**:5052.
9. Nakamura, T. *et al.* (1989) Nature **342**:440.
10. Shiota, G. *et al.* (1995) Hepatology **21**:106.
11. Lindroos, P.M. *et al.* (1991) Hepatology **13**:743.
12. Taman, M. *et al.* (1997) Clin. Nephrol. **48**:241.
13. Seko, Y. *et al.* (2004) Clin. Sci. (London) **106**:439.
14. Kulseng, B. *et al.* (1998) Acta Diabetol. **35**:77.
15. Bell, L.N. *et al.* (2006) Am. J. Physiol. Endocrinol. Metab. **291**:E843.
16. Nakamura, S. *et al.* (1994) Br. J. Haematol. **87**:640.
17. Aune, G. *et al.* (2011) Gynecol. Oncol. **121**:402.
18. Skoldenberg, E.G. *et al.* (2009) Anticancer Res. **29**:3311.
19. Borgiel-Marek, H. *et al.* (2008) Endokrynol. Pol. **59**:467.
20. Osada, S. *et al.* (2008) Hepatogastroenterology **55**:544.
21. Astl, J. *et al.* (2004) Neuro. Endocrinol. Lett. **25**:356.
22. Vessely, D. *et al.* (2004) Physiol. Res. **53**:83.
23. Woodbury, R.L. *et al.* (2002) J. Proteome Res. **1**:233.
24. Feuerherm, A.J. *et al.* (2001) Scand. J. Rheumatol. **30**:229.
25. Grant, D.S. *et al.* (1993) Proc. Natl. Acad. Sci. USA **90**:1937.
26. Bottaro, D.P. *et al.* (1991) Science **251**:802.
27. Giordano, S. *et al.* (1989) Nature **339**:155.
28. Giordano, S. *et al.* (1989) Oncogene **4**:1383.
29. Sakata, H. *et al.* (1997) J. Biol. Chem. **272**:9457.
30. Zioncheck, T.F. *et al.* (1995) J. Biol. Chem. **270**:16871.
31. Klosek, S.K. *et al.* (2005) Biochem. Biophys. Res. Commun. **336**:408.
32. Jo, M. *et al.* (2000) J. Biol. Chem. **275**:8806.
33. Wang, X. *et al.* (2002) Mol. Cell **9**:411.
34. Orian-Rousseau, V. *et al.* (2002) Genes Dev. **16**:3074.
35. Trusolino, L. *et al.* (2001) Cell **107**:643.
36. Giordano, S. *et al.* (2002) Nat. Cell Biol. **4**:720.
37. Conrotto, P. *et al.* (2004) Oncogene **23**:5131.
38. Follenzi, A. *et al.* (2000) Oncogene **19**:3041.
39. Lesko, E. and M. Majka (2008) Front. Biosci. **13**:1271.
40. Sonnenberg, E. *et al.* (1993) J. Cell Biol. **123**:223.
41. Tajima, H. *et al.* (1991) FEBS Lett. **291**:229.
42. Rygaard, K. *et al.* (1993) Int. J. Oncol. **2**:991.
43. Montesano, R. *et al.* (1991) Cell **67**:901.
44. Maeshima, A. *et al.* (2000) Kidney Int. **58**:1511.
45. Dai, C. *et al.* (2003) J. Biol. Chem. **278**:27080.
46. Honda, S. *et al.* (1995) Brain Res. Mol. Brain Res. **32**:197.
47. Ebens, A. *et al.* (1996) Neuron **17**:1157.
48. Benkhoucha, M. *et al.* (2010) Proc. Natl. Acad. Sci. **107**:6424.
49. Rutella, S. *et al.* (2006) Blood **108**:218.
50. Ito, W. *et al.* (2005) Am. J. Respir. Cell. Mol. Biol. **32**:268.

## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.




*All trademarks and registered trademarks are the property of their respective owners.*