Quantikine® ELISA

Mouse/Rat HGF Immunoassay

Catalog Number MHG00

For the quantitative determination of mouse or rat Hepatocyte Growth Factor (HGF) concentrations in cell culture supernates, tissue homogenates, serum, plasma, and platelet-poor plasma.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

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
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	2
TECHNICAL HINTS	2
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	4
SUPPLIES REQUIRED FOR TISSUE HOMOGENATE SAMPLES	4
PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE	5
SAMPLE PREPARATION	5
REAGENT PREPARATION	6
ASSAY PROCEDURE	7
CALCULATION OF RESULTS	8
TYPICAL DATA	8
PRECISION	9
RECOVERY	9
SENSITIVITY	9
CALIBRATION	9
LINEARITY	10
SAMPLE VALUES	11
SPECIFICITY	12
REFERENCES	13
PLATE LAYOUT	14

MANUFACTURED AND DISTRIBUTED BY:

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

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

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). HGF is secreted as an inactive single chain precursor 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 α-chain and a 30 kDa C-terminal β-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). Mouse HGF shares 91-94% amino acid sequence identity with bovine, canine, feline, human, 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 α- and 145 kDa C-terminal β-chains (28). The strictly extracellular α-chain remains disulfide-linked to the β-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 underlies 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® Mouse/Rat HGF Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse or rat HGF in cell culture supernates, tissue homogenates, serum, plasma, and platelet-poor plasma. It contains NS0-expressed recombinant mouse HGF and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant factor accurately. Results obtained using natural HGF showed dose-response 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 mouse or rat HGF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse/rat HGF has been pre-coated onto a microplate. Standards, control, 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 mouse/rat HGF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of HGF bound in the initial step. The sample values are then read off the standard curve.

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, further dilute the samples with 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.
- 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.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse/Rat HGF Microplate	894093	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse/rat 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.*
Mouse/Rat HGF Conjugate	894094	12 mL of a polyclonal antibody specific for mouse/rat HGF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse/Rat HGF Standard	894095	Recombinant mouse HGF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse/Rat HGF Control	894096	Recombinant mouse HGF in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1-38	895301	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a buffered protein base with preservatives. *Use diluted 1:4 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* 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.
- Test tubes for dilution of standards and samples.

SUPPLIES REQUIRED FOR TISSUE HOMOGENATE SAMPLES

- Cell Lysis Buffer 2 (R&D Systems®, Catalog # 895347).
- PBS

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

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 MSDS 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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue Homogenates - Prior to assay, tissues must be homogenized according to the directions in the Sample Values section. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Mouse Platelet-poor Plasma - Collect plasma on ice using EDTA as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 x g for 10 minutes at 2-8 °C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Rat Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 20 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.

Note: *Rat platelet-poor plasma was not available for testing when this assay was developed.*
Heparin plasma samples are not suitable for use in this assay.
Citrate plasma has not been validated for use in this assay.
Hemolyzed and lipemic samples are not suitable for use in this assay.

HGF is present in platelets. Therefore, to measure circulating levels of HGF, platelet-poor plasma should be collected for measurement. It should be noted that many protocols for plasma preparation, including procedures recommended by the Clinical and Laboratory Standards Institute (CLSI), result in incomplete removal of platelets from blood.

SAMPLE PREPARATION

Tissue homogenates require a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

Mouse serum samples require a 5-fold dilution. A suggested 5-fold dilution is 50 µL of sample + 200 µL of Calibrator Diluent RD5-26 (diluted 1:4).

Mouse platelet-poor plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5-26 (diluted 1:4).

Rat serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD5-26 (diluted 1:4).

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse/Rat HGF Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

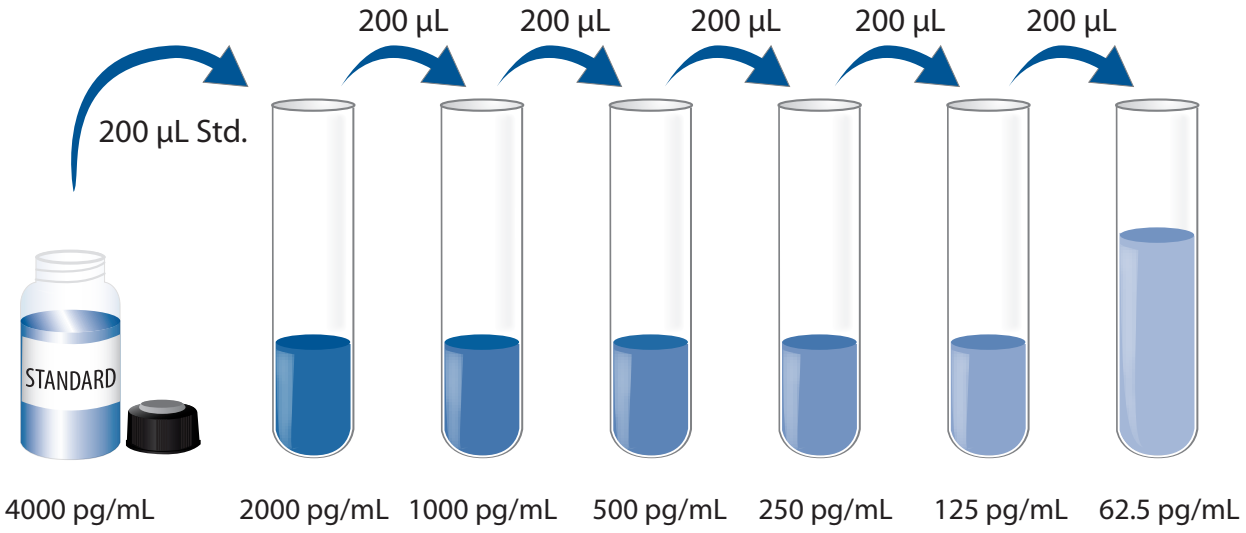
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

Mouse/Rat HGF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse/Rat HGF Standard with Calibrator Diluent RD5-26 (diluted 1:4). This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-26 (diluted 1:4) into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse/Rat Standard (4000 pg/mL) serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 pg/mL).



For research use only. Not for use in diagnostic procedures.

ASSAY PROCEDURE

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

1. Prepare all reagents, working standards, control, and samples 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 50 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. A plate layout is provided to record the standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five 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 100 µL of Mouse/Rat HGF Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

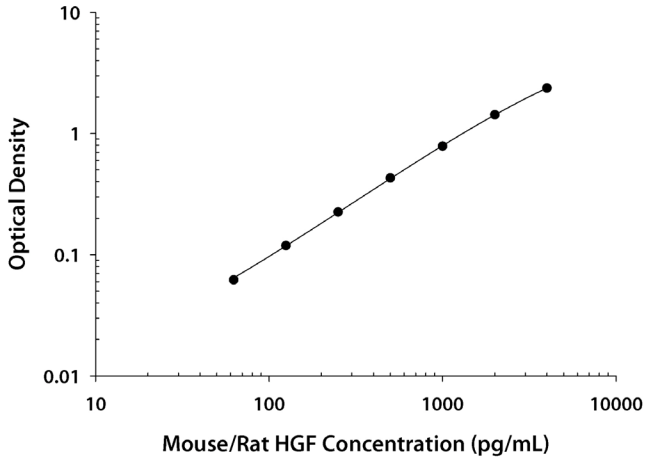
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse/rat 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	0.032 0.032	0.032	—
62.5	0.093 0.095	0.094	0.062
125	0.148 0.154	0.151	0.119
250	0.252 0.262	0.257	0.225
500	0.453 0.471	0.462	0.430
1000	0.814 0.822	0.818	0.786
2000	1.441 1.479	1.460	1.428
4000	2.398 2.407	2.403	2.371

For research use only. Not for use in diagnostic procedures.

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 kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	217	452	1168	218	447	1107
Standard deviation	8.60	19.7	62.2	14.9	21.0	87.7
CV (%)	4.0	4.4	5.3	6.8	4.7	7.9

RECOVERY

The recovery of mouse/rat HGF spiked into various matrices was evaluated.

Mouse Samples	Average % Recovery	Range
Cell culture supernate (n=4)	98	85-106%
Tissue homogenate* (n=3)	87	80-101%
Serum* (n=4)	96	84-112%
Platelet-poor EDTA plasma* (n=4)	104	101-106%

Rat Samples	Average % Recovery	Range
Cell culture supernate (n=4)	102	85-114%
EDTA plasma* (n=4)	108	100-116%

*Samples were diluted prior to assay.

SENSITIVITY

Sixty-six assays were evaluated and the minimum detectable dose (MDD) of mouse/rat HGF ranged from 1.33-12.1 pg/mL. The mean MDD was 4.38 pg/mL.

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

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-derived recombinant mouse HGF produced at R&D Systems® [Gln33-Arg495 (alpha chain) & Val496-Leu728 (beta chain) Accession # Q53WS5].

LINEARITY

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

Mouse		Cell culture supernates (n=4)	Tissue homogenates* (n=4)	Serum* (n=4)	Platelet-poor EDTA plasma* (n=4)
1:2	Average % of Expected	100	104	99	104
	Range (%)	97-103	96-111	95-100	97-106
1:4	Average % of Expected	100	104	96	106
	Range (%)	97-102	95-110	94-98	100-110
1:8	Average % of Expected	99	101	94	105
	Range (%)	94-102	95-112	90-98	99-108
1:16	Average % of Expected	99	100	93	101
	Range (%)	91-104	94-110	88-96	99-103

Rat		Cell culture supernates (n=4)	Serum* (n=4)	EDTA plasma* (n=4)
1:2	Average % of Expected	99	98	105
	Range (%)	96-106	82-105	102-107
1:4	Average % of Expected	97	103	108
	Range (%)	93-103	101-105	102-113
1:8	Average % of Expected	96	108	107
	Range (%)	92-99	107-109	102-115
1:16	Average % of Expected	99	109	109
	Range (%)	95-102	100-115	104-117

*Samples were diluted prior to assay.

For research use only. Not for use in diagnostic procedures.

SAMPLE VALUES

Serum/Plasma - Mouse and rat samples were evaluated for the presence of mouse/rat HGF in this assay.

Mouse Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=20)	11,178	5381-21,185	3678
Platelet-poor EDTA plasma (n=20)	2809	964-6438	1625

Rat Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=16)	32,991	16,491-42,103	7376
EDTA plasma (n=17)	5711	363-17,590	5679

Cell Culture Supernates:
Tissues from mice or rats were removed, rinsed in PBS, and kept on ice in PBS. Tissues were then homogenized using a tissue homogenizer and seeded into media containing RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate, and cultured for the times indicated. Aliquots of the cell culture supernates were removed and assayed for levels of mouse/rat HGF.

Tissue Type	(pg/mL)
Mouse kidney (1 day)	143
Mouse liver (1 day)	164
Mouse spleen (1 day)	395
Rat kidney (18 hours)	2820
Rat spleen (18 hours)	196

3T3-L1 mouse embryonic fibroblast adipose-like cells (2 x 10^5 cell/mL) were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 3 days. Media was then removed from cells. 50 mL of DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, 1 µg/mL bovine insulin, 0.5 mM methylisobutylxanthine, and 1 µM dexamethasone was added to cells and then incubated for 4 days. An aliquot of the cell culture supernate was removed, assayed for mouse HGF, and measured 12,200 pg/mL.

Tissue Homogenates - Organs from mice were rinsed with PBS, cut into 1-2 mm pieces, and homogenized with a tissue homogenizer in PBS. An equal volume of Cell Lysis Buffer 2 was added and tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. Aliquots of the homogenates were removed and assayed for levels of mouse HGF.

Tissue Type	(pg/mL)
Kidney	29,119
Liver	10,745
Spleen	473

SPECIFICITY

This assay recognizes natural and recombinant mouse/rat HGF.

The factors listed below were prepared at 500 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 500 ng/mL in a mid-range recombinant mouse/rat HGF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:
HAI-1
HAI-2B
HGF Activator
Pro-HGF Activator
HGF R/c-MET
MSP

Recombinant human:
HAI-2
HGF
HGF single chain precursor

Recombinant canine:
HGF

Recombinant mouse HGF single chain precursor cross-reacts 100% in this assay.

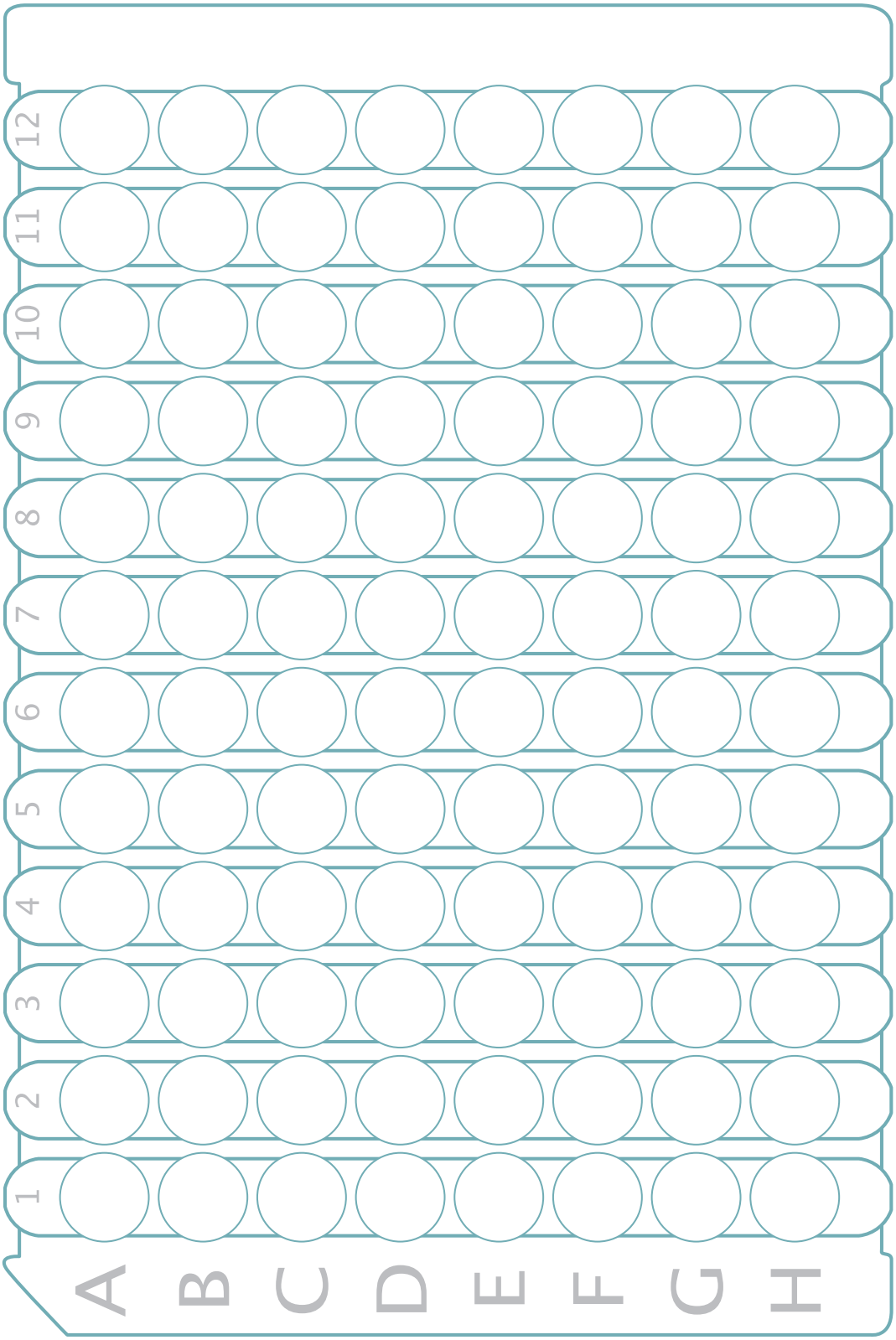
For research use only. Not for use in diagnostic procedures.

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.

©2016 R&D Systems®, Inc.