PRODUCT INFORMATION & MANUAL

Human HGF Instant ELISA

BMS2069INST

Enzyme-linked immunosorbent assay for quantitative detection of human HGF.

For research use only.
Not for diagnostic or therapeutic procedures.
128 Tests

eBioscience®
An Affymetrix Company

Human HGF Instant ELISA

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TABLE OF CONTENTS

1	Intended Use	3
2	Summary	3
3	Principles of the Test	3
4	Reagents Provided	6
5	Storage Instructions	7
6	Specimen Collection	7
7	Materials Required But Not Provided	8
8	Precautions for Use	9
9	Preparation of Reagents and Samples	11
10	Test Protocol	12
11	Calculation of Results	16
12	Limitations	20
13	Performance Characteristics	21
15	Ordering Information	26
16	Reagent Preparation Summary	26
17	Test Protocol Summary	28

1 Intended Use

The human HGF Instant ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of human HGF. **The human HGF Instant ELISA is for research use only. Not for diagnostic or therapeutic procedures**

2 Summary

Hepatocyte growth factor (HGF), also called scatter factor (SF), is a paracrine multifunctional pleiotropic cytokine. HGF is a mesenchymally derived heparin-binding glycoprotein that is secreted as a single-chain (pro-HGF), biologically inert precursor. Under appropriate conditions such as tissue damage, pro-HGF is converted to its bioactive form by proteolytic digestion at a specific site within the molecule. Mature HGF is a heterodimer, consisting of a 69 kDa α- and a 34 kDa ß-chain held together by a single disulfide bond.
HGF is produced mainly in the liver. Kupffer cells play a stimulatory role in liver regeneration by enhancing HGF expression. In addition, studies indicate that HGF accelerates the proliferation of hepatic oval cells and promotes the differentiation to hepatocytes. HGF is furthermore produced in human platelets, kidney, serum, placenta, lung and spleen, and in the HL-60 cell line.
HGF transduces multiple biological effects by binding to the receptor tyrosine kinase encoded by the c-Met proto-oncogene. The HGF receptor (HGFR) is expressed in normal epithelium of almost every tissue. Other cell types such as melanocytes, endothelial cells, microglial cells, neurons, hematopoietic cells, and a variety of tumor cell lines of various origins also express this receptor.

HGF acts on a wide variety of epithelial cells as a mitogen (stimulation of cell growth), a motogen (stimulation of cell motility), and a morphogen (induction of multicellular tissue-like structure). Due to these functions HGF is considered a key molecule for the construction of normal tissue structure during embryogenesis, organogenesis, and organ regeneration (Nakamura).
Potent anti-inflammatory activity has been observed in several animal models and multiple organ systems. HGF suppresses inflammation by targeting multiple pathophysiological processes involved in inflammatory response, including activation of parenchymal cells, microvascular endothelial dysfunction, and leukocyte extravasation and chemotaxis. This anti-inflammatory action functions by intercepting NFkappaB

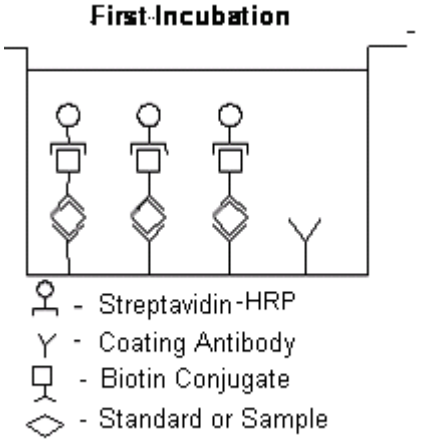
signaling and subsequently disrupting the expression of NFkappaB-dependent proinflammatory mediators.
Furthermore, HGF is constitutively produced by bone marrow stromal cells and is thought to enhance hematopoiesis.
HGF is also a potent stimulator of angiogenesis and cancer metastasis. Through interaction with the c-met receptor HGF may also stimulate chemotaxis and growth of malignant cells. HGF is elevated in serum of liver disease patients, and also in patients with various kinds of cancers.

For literature update refer to **www.eBioscience.com**

3 Principles of the Test

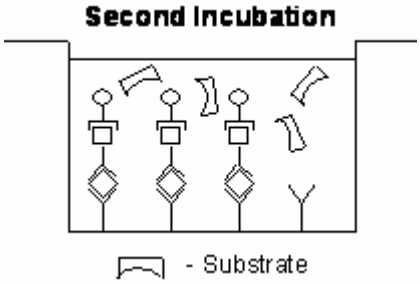
An anti-human HGF coating antibody is adsorbed onto microwells. Human HGF present in the sample or standard binds to antibodies adsorbed to the microwells; a biotin-conjugated anti-human HGF antibody binds to human HGF captured by the first antibody. Streptavidin-HRP binds to the biotin conjugated anti-human HGF.

Figure 1



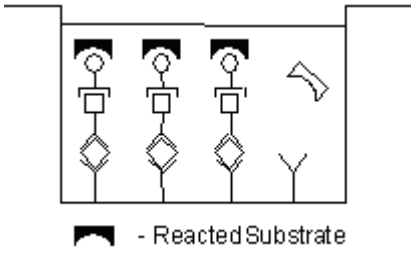
Following incubation unbound biotin conjugated antihuman HGF and Streptavidin-HRP is removed during a wash step, and substrate solution reactive with HRP is added to the wells.

Figure 2



A coloured product is formed in proportion to the amount of soluble human HGF present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 human HGF standard dilutions and human HGF sample concentration determined.

Figure 3



4 Reagents Provided

1 aluminium pouch with a **Microwell Plate coated** with antibody against human HGF, **Biotin-Conjugate** (anti-human HGF antibody), **Streptavidin-HRP** and Sample Diluent, lyophilized

2 aluminium pouches with a human HGF **Standard curve** (coloured)

1 bottle (25 ml) **Wash Buffer Concentrate** 20x (phosphate-buffered saline with 1% Tween 20)

1 vial (15 ml) **Substrate Solution** (tetramethyl-benzidine)

1 vial (12 ml) **Sample Diluent** (Use when an external predilution of the samples is needed)

1 vial (15 ml) **Stop Solution** (1M Phosphoric acid)

2 **Adhesive Films**

5 Storage Instructions

Store ELISA plate and Standard curves or whole kit at -20°C. The plate and the standard curves can also be removed, stored at -20°C, remaining kit reagents can be stored between 2° and 8°C. Expiry of the kit and reagents is stated on labels.
The expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, the reagent is not contaminated by the first handling.

6 Specimen Collection

Cell culture supernatant, serum and plasma (citrate) were tested with this assay. Other biological samples might be suitable for use in the assay. Remove serum or plasma from the clot or cells as soon as possible after clotting and separation.

Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples must be stored frozen at -20°C to avoid loss of bioactive human HGF. If samples are to be run within 24 hours, they may be stored at 2° to 8°C (for sample stability refer to 13).
Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

7 Materials Required But Not Provided

- 5 ml and 10 ml graduated pipettes
- 5 µl to 1000 µl adjustable single channel micropipettes with disposable tips
- adjustable multichannel micropipettes (for volumes between 50 µl and 500 µl) with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microplate shaker
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform linear regression analysis

8 Precautions for Use

- All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statements(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or specimens.
- Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- Avoid contact of substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- In order to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- Use clean, dedicated reagent trays for dispensing substrate reagent.

- Glass-distilled water or deionized water must be used for reagent preparation.
- Substrate solution must be at room temperature prior to use.
- Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

9 Preparation of Reagents and Samples

Buffer concentrate should be brought to room temperature and diluted before starting the test procedure. If crystals have formed in the buffer concentrate, warm it gently until crystals have completely dissolved.

9.1 Wash Buffer (1x)

Pour entire contents (25 ml) of the Wash Buffer Concentrate (20x) into a clean 500 ml graduated cylinder. Bring to final volume to 500 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Please note that Wash Buffer (1x) is stable for 30 days.

10 Test Protocol

- **Use plate immediately after removal from -20°C!**
- **Do not wait until pellets have completely dissolved before applying samples - the binding reaction in the standard strips starts immediately after addition of water!**
- **Do not try to dissolve pellets by pipetting up and down in the wells - some parts of the pellet could stick to the tip creating high variation of results.**
- **Perform the washing step with at least 400 µl of washing buffer as stated in the manual or fill the wells completely - otherwise any pellet residues sticking to the rim of the well will not be removed and create high variation of results.**
- **Allow the washing buffer to sit in the wells for a few seconds before aspiration.**
- **Remove covers of the standard strips carefully so that all the lyophilized pellets remain in the wells.**

a. Determine the number of Microwell Strips required to test the desired number of samples plus Microwell Strips for blanks and standards (coloured). Each sample, standard and blank should be assayed in duplicate. Remove extra Microwell Strips from holder and store in foil bag with the desiccant provided at -20°C sealed tightly. Place Microwell Strips containing the standard curve in position A1/A2 to H1/H2 (see Table1).

b. Add **distilled water** to all **standard and blank wells** as indicated on the label of the standard strips (A1, A2 to H1, H2).

c. Add 100 µl of **distilled water** to the **sample wells**.

Table 1

Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	**2**	**3**	**4**
A	Standard 1 (4000 pg/ml)	Standard 1 (4000 pg/ml)	Sample 1	Sample 1
B	Standard 2 (2000 pg/ml)	Standard 2 (2000 pg/ml)	Sample 2	Sample 2
C	Standard 3 (1000 pg/ml)	Standard 3 (1000 pg/ml)	Sample 3	Sample 3
D	Standard 4 (500 pg/ml)	Standard 4 (500 pg/ml)	Sample 4	Sample 4
E	Standard 5 (250 pg/ml)	Standard 5 (250 pg/ml)	Sample 5	Sample 5
F	Standard 6 (125 pg/ml)	Standard 6 (125 pg/ml)	Sample 6	Sample 6
G	Standard 7 (63 pg/ml)	Standard 7 (63 pg/ml)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

d. Add 50 µl of each **sample**, in duplicate, to the **designated wells** and mix the contents.

e. Cover with an adhesive film and incubate at room temperature (18ºC to 25ºC) for 3 hours on a microplate shaker. **(Shaking is absolutely necessary for an optimal test performance.)**

f. Remove adhesive film and empty wells. **Wash** the microwell strips 6 times with approximately **400 µl** Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about **10 – 15 seconds** before aspiration. Take care not to scratch the surface of the microwells.

   After the last wash, tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing or place upside down on a wet absorbent paper for no longer than 15 minutes. Do not allow wells to dry.

g. Pipette 100 µl of **TMB Substrate Solution** to all wells, including the blank wells.

h. Incubate the microwell strips at room temperature (18° to 25°C) for 30 minutes. Avoid direct exposure to intense light.

   The colour development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for colour development has to be done individually for each assay.

   It is recommended to add the Stop Solution when the highest standard has developed a dark blue colour. Alternatively the colour development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9 – 0.95.

i. Stop the enzyme reaction by quickly pipetting 100 µl of **Stop Solution** into each well, including the blank wells. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2 - 8°C in the dark.

j. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the human HGF standards.

Note: Shaking is absolutely necessary for an optimal test performance.

11 Calculation of Results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 per cent of the mean.

- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human HGF concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).

- To determine the concentration of circulating human HGF for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human HGF concentration.

- **Samples have been diluted 1:2, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 2).**

- **Calculation of samples with a concentration exceeding standard 1 may result in incorrect, low human HGF levels. Such samples require further external predilution according to expected human HGF values with Sample Diluent in order to precisely quantitate the actual human HGF level.**

- It is suggested that each testing facility establishes a control sample of known human HGF concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.

- A representative standard curve is shown in Figure 4. This curve cannot be used to derive test results. Every laboratory must prepare a standard curve for each group of microwell strips assayed.

* N.B: There is a common dilution factor for samples due to the conjugate which must then be included in the calculation. The samples contribute 100 µl to the final volume per well. These 100 µl are composed of 50 µl of Sample Diluent plus 50 µl of the sample. This is a 1:2 dilution.
The remaining 50 µl to give 150 µl are due to the addition of 50 µl conjugate to all wells.
50 µl Sample Diluent and 50 µl conjugate results in 100 µl reconstitution volume, addition of 50 µl sample (50 µl + 50 µl sample = 1:2 dilution).

Figure 4

Representative standard curve for human HGF Instant ELISA. Human HGF was diluted in serial 2-fold steps in Sample Diluent. Each symbol represents the mean of 3 parallel titrations.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

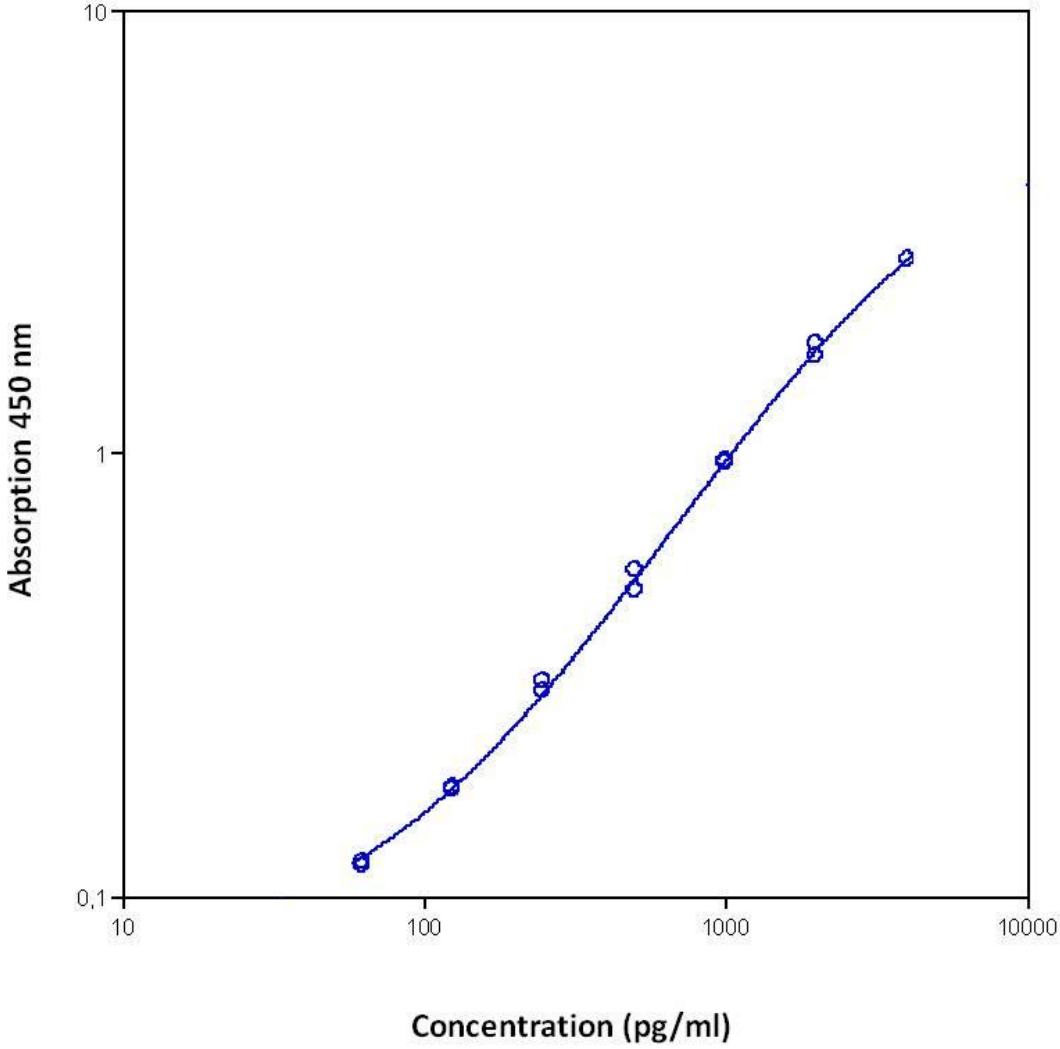


Table 2
Typical data using the human HGF INSTANT ELISA
Measuring wavelength: 450 nm
Reference wavelength: 620 nm

Standard	Human HGF Concentration (pg/ml)	O.D. (450 nm)	O.D. Mean	C.V. (%)
1	4000	2.743	2.744	0.0
		2.745		
2	2000	1.663	1.720	3.3
		1.776		
3	1000	0.967	0.961	0.6
		0.956		
4	500	0.545	0.518	5.1
		0.492		
5	250	0.307	0.299	2.8
		0.290		
6	125	0.174	0.176	0.8
		0.177		
7	63	0.118	0.119	1.0
		0.120		
Blank	0	0.032	0.032	0.2
		0.032		

The OD values of the standard curve may vary according to the conditions of assay performance (e.g. operator, pipetting technique, washing technique or temperature effects). Furthermore shelf life of the kit may affect enzymatic activity and thus colour intensity. Values measured are still valid.

12 Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.
- The use of radioimmunotherapy has significantly increased the number of patients with human anti-mouse IgG antibodies (HAMA). HAMA may interfere with assays utilizing murine monoclonal antibodies leading to both false positive and false negative results. Serum samples containing antibodies to murine immunoglobulins can still be analysed in such assays when murine immunoglobulins (serum, ascitic fluid, or monoclonal antibodies of irrelevant specificity) are added to the sample.

13 Performance Characteristics

13.1 Sensitivity

The limit of detection of human HGF defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 5.5 pg/ml (mean of 4 independent assays).

13.2 Reproducibility

13.2.1 Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 7 serum samples containing different natural levels of human HGF. 2 standard curves were run on each plate. Data below show the mean human HGF concentration and the coefficient of variation for each sample (see Table 3). The calculated overall intra-assay coefficient of variation was 7.6%.

Table 3

The mean human HGF concentration and the coefficient of variation for each sample.

Positive Sample	Experiment	Human HGF Concentration (pg/ml)	Coefficient of Variation (%)
1	1	3526.6	6.0
	2	3730.2	5.9
	3	4129.0	9.4
2	1	2473.4	5.0
	2	2652.7	7.8
	3	2725.9	4.4
3	1	1720.6	7.1
	2	1900.0	9.6
	3	1759.1	7.2
4	1	1047.4	8.5
	2	1285.9	11.1
	3	1161.7	6.4
5	1	424.5	3.8
	2	511.0	7.4
	3	424.4	12.1
6	1	313.4	4.1
	2	339.6	7.0
	3	292.6	12.7
7	1	323.2	6.3
	2	336.8	7.8
	3	395.8	9.4

13.2.2 Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of 7 serum samples containing different concentrations of human HGF. 2 standard curves were run on each plate. Data below show the mean human HGF concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 8.3%.

Table 4
The mean human HGF concentration and the coefficient of variation of each sample

Sample	Mean human HGF Concentration (pg/ml)	Coefficient of Variation (%)
1	3795	8.1
2	2617	5.0
3	1793	5.3
4	1165	10.2
5	453	11.0
6	315	7.5
7	352	11.0

13.3 Spike Recovery

The spike recovery was evaluated by spiking 3 levels of human HGF into serum, plasma (citrate) and cell culture supernatant. Recoveries were determined with 4 replicates each. The amount of endogenous human HGF in unspiked samples was subtracted from the spike values.

For recovery data see Table 5.

Table 5

Sample matrix	Spike high		Spike medium		Spike low	
	Mean (%)	Range (%)	Mean (%)	Range (%)	Mean (%)	Range (%)
Serum	86	76 – 93	98	88 – 114	105	98 – 117
Plasma (citrate)	103	92 – 115	107	92 – 119	98	82 – 104
Cell culture supernatant	92	79 – 85	88	86 – 90	90	73 – 106

13.4 Dilution Parallelism

Serum, plasma (citrate) and cell culture supernatant samples with different levels of human HGF were analysed at serial 2-fold dilutions with 4 replicates each.

For data see Table 6.

Table 6

Sample matrix	Recovery of Exp. Val.		
	Range (%)	Mean (%)	Range (%)
Serum	1:4 1 :8 1 :16	99 109 115	96 – 102 93 – 125 110 – 126
Plasma (citrate)	1:4 1 :8 1 :16	89 105 111	79 – 106 88 – 138 93 – 152
Cell culture supernatant	1:4 1 :8 1 :16	95 108 110	93 – 97 108 103 – 116

13.5 Sample Stability

13.5.1 Freeze-Thaw Stability

Aliquots of serum samples (unspiked or spiked) were stored at -20°C and thawed 3 times, and the human HGF levels determined.
There was no significant loss of human HGF immunoreactivity detected by freezing and thawing.

13.5.2 Storage Stability

Aliquots of serum samples (spiked or unspiked) were stored at -20°C, 2-8°C and room temperature (RT), and the human HGF level determined after 24 h. There was no significant loss of human HGF immunoreactivity detected during storage under above conditions.

13.6 Specificity

The assay detects both natural and recombinant human HGF.
The interference of circulating factors of the immune syteme was evaluated by spiking these proteins at physiologically relevant concentrations into positive serum.
There was no crossreactivity detected with EGF, VEGF-A, PDGF-BB, VEGF-C, PDGF-AA, KDR and VEGF-R1.

14 Ordering Information

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15 Reagent Preparation Summary

15.1 Wash Buffer (1x)

Add **Wash Buffer Concentrate** 20x (25 ml) to 475 ml distilled water

16 Test Protocol Summary

- Place standard strips in position A1/A2 to H1/H2.
- Add distilled water, in duplicate, to all standard and blank wells as indicated on the label of the standard strips.
- Add 100 µl distilled water to sample wells.
- Add 50 µl sample to designated wells.
- Cover microwell strips and incubate 3 hours at room temperature (18° to 25°C) on a microplate shaker. (Shaking is absolutely necessary for an optimal test performance).
- Empty and wash microwell strips 6 times with 400 µl Wash Buffer.
- Add 100 µl of TMB Substrate Solution to all wells including blank wells.
- Incubate the microwell strips for 30 minutes at room temperature (18° to 25°C).
- Add 100 µl Stop Solution to all wells including blank wells.
- Blank microwell reader and measure colour intensity at 450 nm.

Note: Samples have been diluted 1:2, thus the concentration read from the standard curve must be multiplied by the dilution factor (x 2)