PRODUCT INFORMATION & MANUAL

Human HGF Valukine™ ELISA

Catalog Number: VAL168

For the quantitative determination of natural and recombinant human HGF concentrations

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

Bio-Techne China Co. Ltd
P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001
info.cn@bio-techne.com

Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version 202403.2

TABLE OF CONTENTS

I. BACKGROUND 2

II. OVERVIEW 4

III. ADVANTAGES 5

IV. EXPERIMENT 7

V. KIT COMPONENTS AND STORAGE 8

VI. PREPARATION 11

VII. ASSAY PROCEDURE 13

VIII. REFERENCES 15

I. BACKGROUND

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 disulfidelinked 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).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human HGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human HGF present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for human HGF is pipetted into the wells. After washing away any unbound substances, Streptavidin-HRP is pipetted into the wells. Following a wash to remove any unbound reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human HGF bound in the initial step. The color development is stopped, and the intensity of the color is measured.

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernate and human serum.
- 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 Reagent Diluent (1×) or Calibrator Diluent-S (1×) and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

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

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	240.8	981.8	3810.9	250.5	1012.2	3998.7
Standard Deviation	11.1	28.1	119.4	17.3	51.7	321.8
CV%	4.6	2.9	3.1	6.9	5.1	8.0

B. RECOVERY

The recovery of human HGF spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 95.1 to 123.1% with an average of 112.7%.

The recovery of human HGF spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 114.0 to 125.9% with an average of 121.0%.

C. SENSITIVITY

The minimum detectable dose (MDD) of human HGF is typically less than 9.18 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.

D. CALIBRATION

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

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human HGF and diluted with Reagent Diluent 1× (*for cell culture supernate samples*) or Calibrator Diluent-S 1× (*for serum samples*) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	90.2	86.2-96.7
1:4	89.6	82.8-101.9
1:8	91.0	80.3-108.1
1:16	89.6	80.2-103.4

F. SAMPLE VALUES

Human serum - Eight human serum samples were evaluated for the presence of human HGF in this assay. All samples measured ranged from 1308.2 to 1846.6 pg/mL with an average of 1632.0 pg/mL.

G. SPECIFICITY

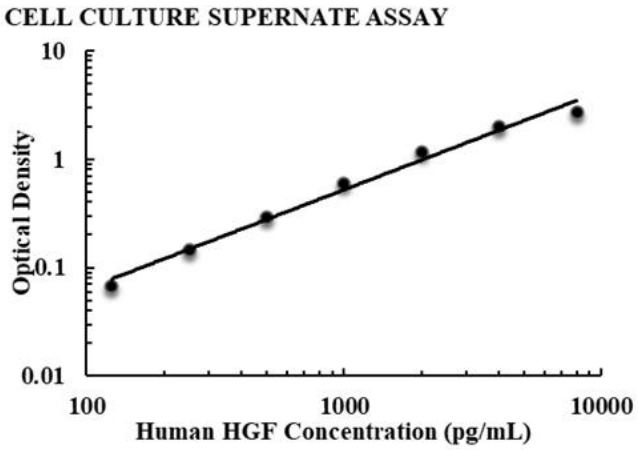
The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity but do interfere in this assay.

Recombinant human	Recombinant Mouse
HGF R	HGF R
MSP	

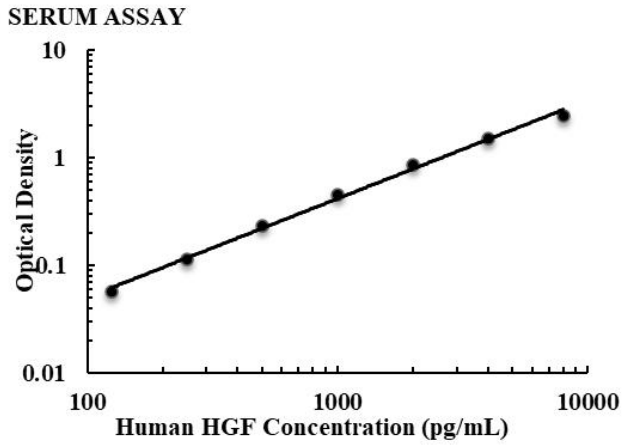
IV. EXPERIMENT

EXAMPLE STANDARD

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



pg/mL	OD	Average	Corrected
0	0.030 0.034	0.032	-
125	0.100 0.100	0.100	0.068
250	0.176 0.176	0.176	0.144
500	0.321 0.327	0.324	0.292
1000	0.617 0.621	0.619	0.587
2000	1.161 1.190	1.176	1.144
4000	2.016 2.041	2.029	1.997
8000	2.735 2.739	2.737	2.705



pg/mL	OD	Average	Corrected
0	0.031 0.032	0.032	-
125	0.087 0.089	0.088	0.057
250	0.142 0.147	0.145	0.113
500	0.259 0.264	0.262	0.230
1000	0.480 0.485	0.483	0.451
2000	0.885 0.900	0.893	0.861
4000	1.488 1.591	1.540	1.508
8000	2.436 2.494	2.465	2.434

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human HGF Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human HGF.	1 plate
Human HGF Standard	Recombinant human HGF in a buffered protein base, lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human HGF Detection Antibody	Biotinylated human HGF antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Streptavidin-HRP A (200×)	200× Streptavidin conjugated to horseradish peroxidase.	1 vial
Reagent Diluent Concentrate (10×)	A 10× concentrated buffered base used to dilute standard, samples, Detection Antibody and HRP.	1 vial
Calibrator Diluent-S Concentrate (2×)	Concentrated buffer diluent used to dilute standard and samples.	1 vial
Detection Antibody Diluent Concentrate (4×)	A 4× concentrated buffered protein base used to dilute Detection Antibody.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution.	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Sealers	Adhesive strip.	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8 °C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="9">Opened/ Reconstituted Reagents</td><td>Streptavidin-HRP A</td><td rowspan="4">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Wash Buffer (1×)</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Standard</td><td>Prepare fresh for each assay.
Standards may be stored for up to 1 month at -20 °C.*</td></tr>
<tr><td>Detection Antibody</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Reagent Diluent Concentrate (10×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Calibrator Diluent -S Concentrate (2×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent-S (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Detection Antibody Diluent Concentrate (4×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Detection Antibody Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*</td></tr>
</table>

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

C. 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.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker capable of maintaining a speed of 500±50 rpm.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- The Stop Solution provided with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.
- Human HGF is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Reagent Diluent (1×).

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. Samples may require dilution with Calibrator Diluent-S (1×).

B. SAMPLE PREPARATION

Human serum samples recommend a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of **Calibrator Diluent-S (1×).** Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.
Note: human HGF are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.

Wash Buffer (1×)- 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 (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

Calibrator Diluent-S (1×) - Use deionized or distilled water to prepare Calibrator Diluent-S (1×).

Reagent Diluent (1×) - Use deionized or distilled water to prepare Reagent Diluent (1×).

Detection Antibody Diluent (1×) - Use deionized or distilled water to prepare Detection Antibody Diluent (1×).

Detection Antibody (1×)- Centrifuge briefly before opening. Reconstitution Volume refer to vial label to prepare Detection Antibody (100×). Allow the Detection Antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Aliquot and store if needed. Dilute to Detection Antibody (1×) with Detection Antibody Diluent (1×). Prepare at least 15 minutes prior to use.

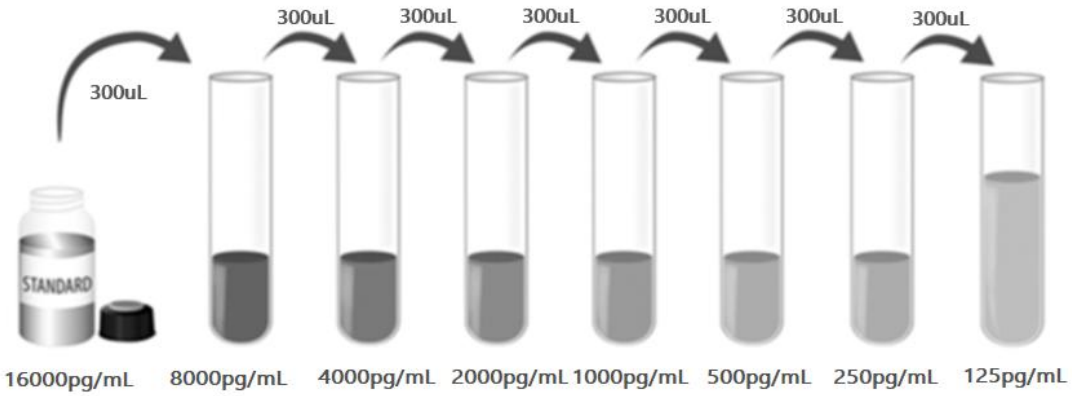
Streptavidin-HRP A (1×) - Centrifuge briefly before opening. Dilute to the working

concentration specified on the vial label using Reagent Diluent (1×).

Human HGF Standard- Centrifuge briefly before opening. Refer to the vial label for the reconstitution volume*. Reconstitute the human HGF Standard with **Reagent Diluent (1×)** (*for cell culture supernate samples*) or **Calibrator Diluent-S (1×)** (*for serum samples*). This reconstitution produces a stock solution of 16000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

*If you have any question, please seek help from our Technical Support.

Pipette 300 µL of **Reagent Diluent (1×)** or **Calibrator Diluent-S (1×)** into each 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 **Reagent Diluent (1×)** or **Calibrator Diluent-S (1×)** serves as the zero standard (0 pg/mL).



D. 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.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB Substrate. 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 TMB Substrate.

VII.ASSAY PROCEDURE

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

Note: human HGF are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.

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 standard and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500±50 rpm.** A plate layout is provided for a record of standards and samples assayed. (Samples may require dilution. See Sample Preparation section.)
4. 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.
5. Add 100 µL of the Detection Antibody (1×) diluted in Detection Antibody Diluent (1×) to each well. **Cover with a new adhesive strip and incubate for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500±50 rpm.**
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working dilution of Streptavidin-HRP A to each well. **Cover the plate and incubate for 20 minutes at room temperature on a horizontal orbital microplate shaker set at 500±50 rpm. Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of TMB Substrate to each well. **Incubate for 10 minutes at room temperature on a horizontal orbital microplate shaker set at 500±50 rpm. Protect from light.**

10. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well within 10 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.
12. **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.

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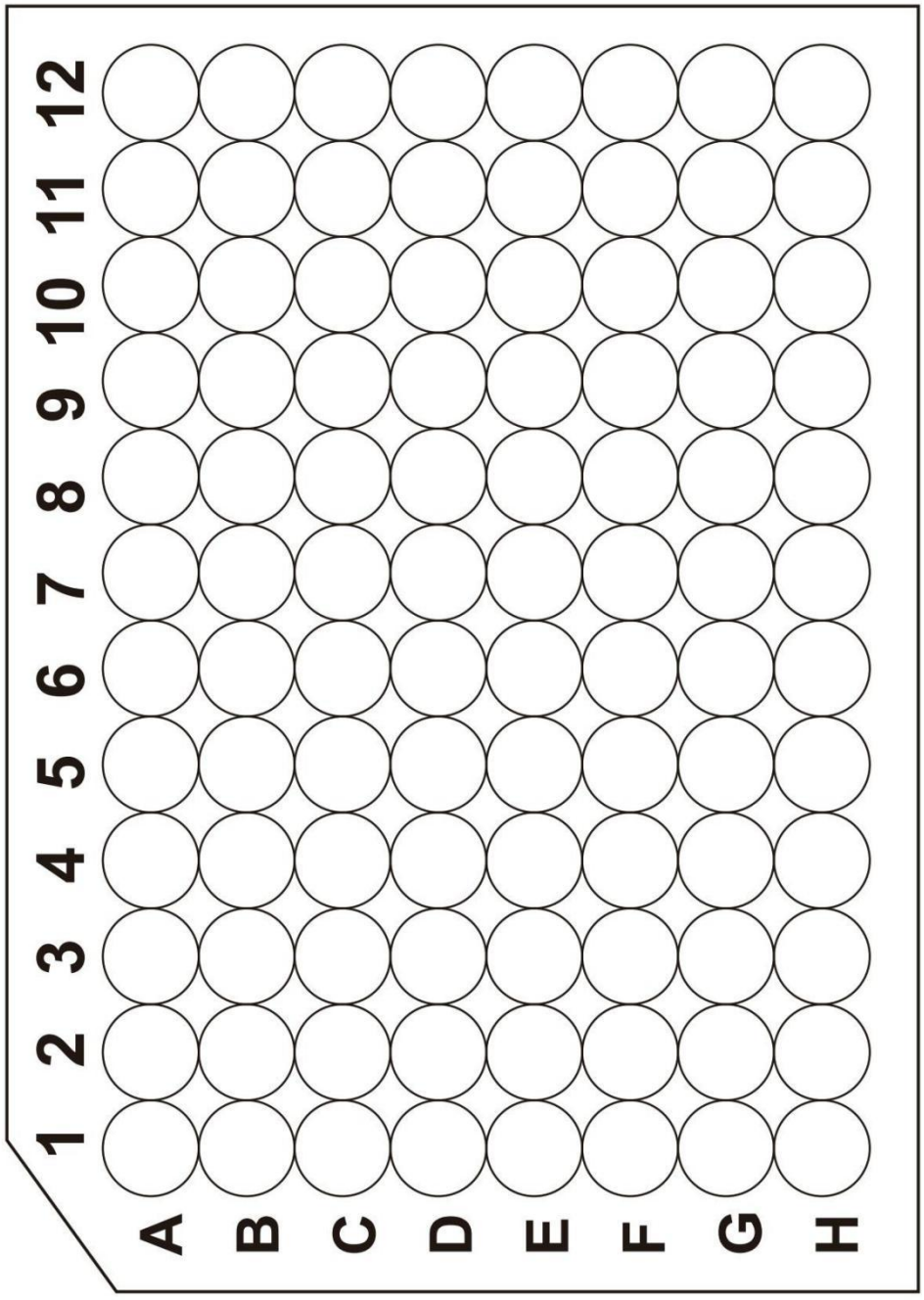
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 HGF Valukine™ ELISA 试剂盒

目录号：VAL168

适用于定量检测天然和重组人 HGF 的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001

info.cn@bio-techne.com

有效期详见试剂盒包装标签

Novus 试剂盒确保在你收货日期 3 个月内有效

版本号 202403.2

目录

I. 背景 20

II. 概述 21

III. 优势 22

IV. 实验 24

V. 试剂盒组成及储存 25

VI. 实验前准备 27

VII. 操作步骤 29

VIII. 参考文献 30

I. 背景

肝细胞生长因子（HGF），也称为分散因子、肝细胞生成素A和乳腺生长因子，是一种调节多种细胞类型生长和迁移的多效性糖蛋白。其结构类似于S1肽酶纤溶酶原。HGF包含一个N-末端PAN/APPLE样结构域、四个Kringle结构域和一个催化失活的丝氨酸蛋白酶样结构域（1，2）。选择性剪接产生的人类HGF亚型缺乏蛋白酶样结构域和不同数量的Kringle结构域。HGF作为非活性单链前肽分泌，可作为可溶性分子循环或与细胞外基质结合（3，4）。在组织损伤部位，前肽在第四个Kringle结构域后被包括HGF激活剂和uPA在内的丝氨酸蛋白酶切割（4-8）。产生的生物活性HGF由60kDa N端α链和30kDa C端β链的二硫键连接的异二聚体组成（4、5、9）。HGF的血清水平在广泛的病理现象中都会升高，包括肝损伤（10、11）、急性肾功能衰竭（12）、心肌梗死（13）、1型糖尿病（14）、肥胖（15）和癌症（16-23），以及类风湿性关节炎患者的滑液（24）。人HGF与牛、犬、猫、小鼠和大鼠HGF具有91-94%的氨基酸序列同源性。HGF表现出明显的物种交叉反应（25）。

HGF通过广泛表达的受体酪氨酸激酶HGF R/c-MET发挥其生物活性（26，27）。该受体经历N-连接糖基化，然后蛋白水解裂解为50 kDa N-末端α链和145 kDa C-末端β链（28）。完全的细胞外α链仍然与β链二硫键相连，β链包含剩余的细胞外、跨膜和细胞质结构域（26、27）。HGF还结合硫酸乙酰肝素蛋白多糖，这些相互作用增强了HGF结合和激活HGF R的能力（29，30）。在没有配体的情况下，HGF R与多种膜蛋白形成非共价复合物，包括CD44v6、CD151、EGF R、Fas、Integrin α6/β4、Plexins B1、B2、B3和MSP R/Ron（31-38）。一种复合物组分的连接可以触发另一种的激活，然后是协同信号效应（31-38）。一些异聚体复合物的形成是上皮细胞形态发生和肿瘤细胞侵袭的必要条件（34-36）。HGF R的过度表达和替代形式的产生与许多人类癌症的发生有关（39）。

HGF由成纤维细胞、脂肪细胞、平滑肌细胞和内皮细胞表达（1）。另一方面，HGF R的表达主要在上皮细胞上发现，表明HGF以旁分泌方式介导基质细胞和上皮细胞之间的相互作用（40）。HGF诱导上皮细胞以及多种其他细胞类型的增殖和迁移，包括肝细胞、软骨细胞、角质形成细胞、黑素细胞和内皮细胞（1）。它对大多数肿瘤细胞作用有丝分裂，但在某些情况下可以反过来抑制其增殖（39、41、42）。在器官发生、组织修复和血管生成过程中，HGF通过诱导细胞散射和分支小管发生促进上皮/内皮形态发生（1、25、43、44）。HGF调节血管生成和上皮细胞运动的能力是其在实体瘤发展中的重要基础（39）。除了形态发生效应外，HGF还可在多种组织中诱导一系列反应（1）。它支持胰岛细胞的存活、增殖和胰岛素分泌（45）。在发育过程中和对损伤的反应中，它作为一种神经营养因子发挥作用（46，47）。它还通过诱导树突状细胞耐受、Treg诱导和Th2偏向来抑制炎症，同时抑制T细胞活化、IL-17表达和炎性细胞浸润（48-50）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人HGF抗体包被于微孔板上，样品和标准品中的人HGF会与固定在板上的抗体结合，游离的成分被洗去；接着加入生物素化的抗人HGF检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶（Streptavidin-HRP）孵育。洗涤去除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合的目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- **仅供科研使用，不可用于体外诊断；**
- 该试剂盒适用于细胞培养上清样本和人血清样本；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用试剂稀释液（1×）或标准品稀释液-S（1×）稀释后重新检测；
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板中分别检测20次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	240.8	981.8	3810.9	250.5	1012.2	3998.7
标准差	11.1	28.1	119.4	17.3	51.7	321.8
CV%	4.6	2.9	3.1	6.9	5.1	8.0

B. 回收率

在细胞培养基样本中掺入检测范围内不同水平的人HGF，测定其回收率。回收率范围在95.1-123.1%，平均回收率在112.7%。

在人血清样本中掺入检测范围内不同水平的人HGF，测定其回收率。回收率范围在114.0-125.9%，平均回收率在121.0%。

C. 灵敏度

人HGF的最低可测量（MDD）一般小于9.18 pg/mL。

MDD是根据20个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

此ELISA试剂盒经由R&D Systems生产的*Sf* 21表达的高纯度重组人HGF蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人HGF，然后用试剂稀释液（1×）（*用于细胞上清样本*）或标准品稀释液-S（1×）（*用于人血清样本*）将样本稀释到检测范围内，测定其线

性。

稀释倍数	平均值 (%)	范围 (%)
1:2	90.2	86.2-96.7
1:4	89.6	82.8-101.9
1:8	91.0	80.3-108.1
1:16	89.6	80.2-103.4

F. 样本预值

人血清样本 - 使用本试剂盒检测了8份人血清样本中人HGF的水平。所有样本的检测值范围为1308.2-1846.6 pg/mL，平均值为1632.0 pg/mL。

G. 特异性

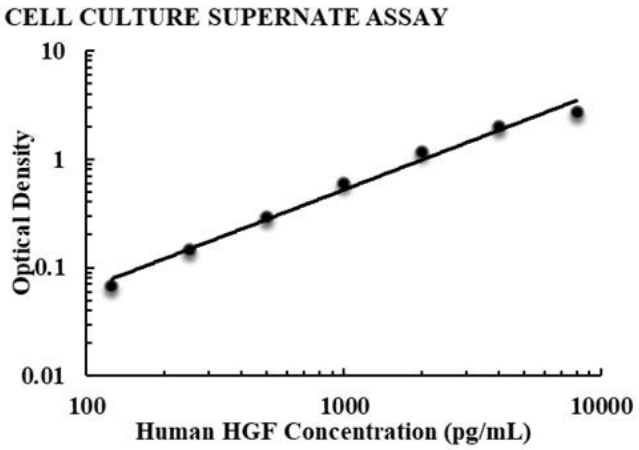
将以下因子配制成50 ng/mL的浓度来检测没有观察到明显的交叉反应，但有干扰。

Recombinant human	Recombinant mouse
HGF R	HGF R
MSP	

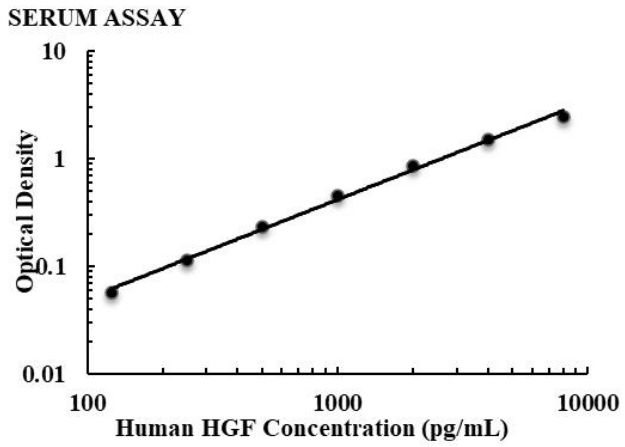
IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



pg/mL	OD	Average	Corrected
0	0.030 0.034	0.032	-
125	0.100 0.100	0.100	0.068
250	0.176 0.176	0.176	0.144
500	0.321 0.327	0.324	0.292
1000	0.617 0.621	0.619	0.587
2000	1.161 1.190	1.176	1.144
4000	2.016 2.041	2.029	1.997
8000	2.735 2.739	2.737	2.705



pg/mL	OD	Average	Corrected
0	0.031 0.032	0.032	-
125	0.087 0.089	0.088	0.057
250	0.142 0.147	0.145	0.113
500	0.259 0.264	0.262	0.230
1000	0.480 0.485	0.483	0.451
2000	0.885 0.900	0.893	0.861
4000	1.488 1.591	1.540	1.508
8000	2.436 2.494	2.465	2.434

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human HGF Microplate	包被抗人 HGF 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human HGF Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Human HGF Detection Antibody	生物素化的人 HGF 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Streptavidin-HRP A (200×)	200×浓缩的链霉亲和素标记的 HRP	1 瓶
Reagent Diluent Concentrate (10×)	浓缩的试剂稀释液（10×）用于稀释标准品、样本、检测抗体、HRP	1 瓶
Calibrator Diluent-S Concentrate (2×)	浓缩的标准品稀释液-S（2×）用于稀释标准品、样本	1 瓶
Detection Antibody Diluent Concentrate (4×)	浓缩的检测抗体稀释液（4×）用于稀释检测抗体	1 瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1 瓶
TMB Substrate	TMB ELISA 底物溶液/TMB 底物溶液	1 瓶
Stop Solution	终止液	1 瓶
Plate Sealers	封板膜	3 张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>链霉亲和素-HRP A</td><td rowspan="4">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤液（1×）</td></tr>
<tr><td>TMB 底物溶液</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*
标准品-20℃储存，最多 30 天*</td></tr>
<tr><td>检测抗体</td><td>分装， -20℃储存，最多 30 天*</td></tr>
<tr><td>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×试剂稀释液</td></tr>
<tr><td>标准品稀释液-S（2×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×标准品稀释液-S</td></tr>
<tr><td>检测抗体稀释液（4×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×检测抗体稀释液</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封：2-8℃储存，最多 30 天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量450 nm检测波长的吸收值及540 nm或570 nm校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 500 mL量筒
- 振荡器（速度可调至500±50 rpm）

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。使用后请彻底洗手。
- 唾液中含有人HGF，为防止试剂盒在检测过程中产生污染，请采取防护措施。

VI. 实验前准备

A. 样品收集及储存

细胞培养上清液：颗粒物应离心去除；立刻检测样本。样本收集后若不及时检测，需按一次使用量分装，冻存于≤-20℃冰箱内，避免反复冻融。样本可能需要用试剂稀释液（1×）稀释。

血清样本: 用血清分离管(SST)分离血清。使血样室温凝集30分钟，然后1000 x g离心15分钟。吸取血清样本之后即刻用于检测，或者分装，≤-20℃贮存备用。避免反复冻融。样本可能需要用标准品稀释液-S（1×）稀释。

B. 样本准备工作

人血清样本建议用标准品稀释液-S（1×）2倍稀释后进行检测，例如：100 µL血清样本+100 µL**标准品稀释液-S（1×）**。最佳稀释度应由最终用户确定。

C. 检测前准备工作

使用前请将所有试剂放置于室温
注：*唾液中含有高浓度的人HGF，为避免污染，实验时请带口罩、手套。*

洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用去离子水或蒸馏水稀释配制成500 mL工作浓度的洗涤液（1×）。

标准品稀释液-S（1×）：使用去离子水或蒸馏水稀释配制成标准品稀释液-S（1×）。

试剂稀释液（1×）：使用去离子水或蒸馏水稀释配制成试剂稀释液（1×）。

检测抗体稀释液（1×）：使用去离子水或蒸馏水稀释配制成检测抗体稀释液（1×）。

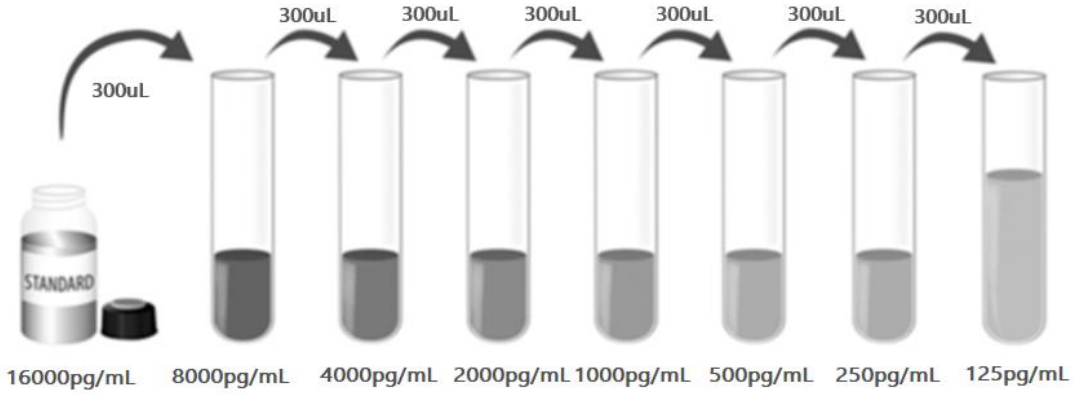
检测抗体（1×）：开盖前请瞬时离心。参考检测抗体瓶标签重溶冻干粉，制备检测抗体（100×）。轻轻震摇至少15分钟，使其充分溶解。如有需要分装保存。再用检测抗体稀释液（1×）稀释至检测抗体（1×），至少在使用前15分钟准备。

链霉亲和素- HRP A（1×）：开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素- HRP A稀释至工作浓度。

人HGF标准品：开盖前请瞬时离心。冻干标准品的重溶体积请参考瓶身标签，用**试剂稀释液（1×）**（*用于细胞上清样本*）或**标准品稀释液-S（1×）**（*用于血清样本*）重溶冻干标准品。得到浓度为16000 pg/mL标准品母液。轻轻震摇至少15分钟，使其充分溶解。

*如有疑问，请咨询我们的技术支持。

向各稀释管中加入300 μL试剂稀释液（1×）或标准品稀释液-S（1×）。将标准品母液参照下图做系列稀释，每管须充分混匀后再移液到下一管。8000 pg/mL管作标准曲线最高点，试剂稀释液(1×)或标准品稀释液- s (1×) 作为零标准（0 pg/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配制不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议15分钟内完成一块板的上样；
- 每次孵育时，正确使用封板膜可保证结果的准确性；
- TMB底物溶液在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- 终止液上板顺序应同TMB底物溶液上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀，请充分混合。

VIII. 操作步骤

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测

注：*唾液中含有高浓度的人HGF，为避免污染，实验时请带口罩、手套。*

1. 按照上一节的说明，准备好所有需要的试剂和标准品；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 分别将不同浓度标准品和实验样本加入相应孔中，每孔100 µL。用封板膜封住反应孔，**室温500±50 rpm水平振荡孵育2小时。**说明书提供了一张96孔模板图，可用于记录标准品和试验样本的板内位置；（样本需要稀释，详情参见样本制备部分。）
4. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液400 µL，然后将板内洗涤液吸去。重复操作3次，共洗4次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体；
5. 在每个微孔内加入100 µL配制好的检测抗体（1×）。用封板膜封住反应孔，**室温500±50 rpm水平振荡孵育2小时**；
6. 重复第4步洗板操作；
7. 在每个微孔内加入100 µL稀释好的链霉亲和素- HRP A工作液。用封板膜封住反应孔，**室温500±50 rpm水平振荡孵育20分钟，注意避光；**
8. 重复第4步洗板操作；
9. 在每个微孔内加入100 µL TMB底物溶液，**室温500±50 rpm水平振荡孵育10分钟，注意避光**；
10. 在每个微孔内加入50 µL终止液，请轻拍微孔板，使溶液混合均匀；
11. 加入终止液后10分钟内，使用酶标仪测量450 nm的吸光度值，设定540 nm或570 nm作为校正波长。如果波长校正不可用，以450 nm的读数减去540 nm或570 nm的读数。这种减法将校正酶标板上的光学缺陷。没有校正而直接在450 nm处进行的读数可能会更高且更不准确；
12. **计算结果：**将每个标准品和样品的复孔吸光值取平均值，然后减去零标准品平均OD值（O.D.），使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。另一替代方法是，通过绘制y轴上每个标准品的平均吸光值与x轴上的浓度来构建标准曲线，并通过图上的点绘制最佳拟合曲线。数据可以通过绘制人HGF浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。该程序将产生足够但不太精确的数据拟合。

    如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

IX. 参考文献

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