PRODUCT INFORMATION & MANUAL

Human KGF/FGF-7 Valukine™ ELISA

Catalog Number: VAL209

For the quantitative determination of natural and recombinant human Keratinocyte Growth Factor (KGF) concentrations

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

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

Version 202410.1

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I. BACKGROUND

Human Keratinocyte Growth Factor (KGF) is a single chain, heparin-binding, 28 kDa glycoprotein that was originally isolated from media conditioned by the growth of human embryonic lung fibroblasts (1, 2). Also known as FGF-7 (or fibroblast growth factor 7), it is part of the rapidly- expanding fibroblast growth factor family that currently includes 14 members (3-6). Mature KGF is 163 amino acid (aa) residues in length, and contains five cysteines, which are not necessary for mitogenic activity, but do contribute to heparin binding (2, 7). Within the FGF family, KGF shows 29% aa sequence identity to FGF-2 and 38% aa sequence identity to FGF-3 (8). Cells reported to express KGF are fibroblasts (1, 9), embryonic mesenchymal cells (10-12), and smooth muscle cells (13).

The receptor for KGF (KGF R) is a restricted-expression splice variant of the bek (bacterially- expressed kinase) gene product, a cell surface receptor with tyrosine kinase activity, also designated FGF R2 (FGF Receptor 2) (14-16). FGF R2 as a full-length, unspliced (or standard), 135 kDa, type I (extracelluar N-terminus) transmembrane glycoprotein with an extracellular domain containing three Ig-like domains plus a heparin-binding motif in the interdomain sequence that connects the N-terminal (D1) and middle (D2) Ig-domains (16). This standard receptor form is expressed ubiquitously in connective tissue cells (17) and binds FGF-1, FGF-2, and FGF-4 with high affinity (Kd ~ 100 pM). FGF-5 and FGF-9 also bind, but with lower affinity (Kd ~2 nM) (16, 18-20). The KGF R splice variant differs from the standard receptor only within a 49 aa residue sequence found in the third (or membrane proximal) Ig-like domain (D3) (15, 21). Although this change has little effect on FGF-1 binding (Kd = 600 pM), its presence decreases FGF-2 binding (Kd = 3 nM) (15) and allows for KGF binding (Kd = 200 pM). As with KGF, the number of cells expressing KGF R are few and limited to epithelial cell types such as keratinocytes (22), transitional epithelium (but not umbrella cells) (23), gastric columnar epithelial cells (24), embryonic lung epithelium (10), mammary epithelium (25), and hepatocytes (26). In addition to the KGF R, KGF also binds to heparan sulfate proteoglycans (HSPG). In general, the role that HSPGs play in the mediation of the biological activities of FGFs is unclear, although they are thought to facilitate binding of FGFs to their high-affinity tyrosine kinase receptors. It has been suggested that HSPGs may hold two FGF molecules in close proximity, thus allowing two individual FGF-FGF R complexes to dimerize or, alternatively, form one FGF-HSPG complex that can actively bind two separate FGF receptors (16). For KGF in particular, however, the HSPGs have been

found to have either no effect or an inhibitory effect on KGF activity. Thus no concensus exists concerning the importance of heparan sulfate for KGF binding and biological activity (27).

Functionally, KGF has been suggested to be a paracrine effector for a number of different epithelial cell types (1, 10, 11, 26, 27). Synthesized by dermal or lamina propria fibroblasts, it is proposed to act locally on the overlying epithelial sheet. In addition to its ability to induce cell proliferation (27), it may also promote epithelial differentiation (12). During wound healing, KGF's role as a re-epithelializing agent is complemented by the presence of proinflammatory molecules which appear as a result of tissue damage. Cytokines such as IL-1(α and β) and IL-6 not only activate local connective tissue cells, resulting in foreign body clearance and tissue remodeling, but also stimulate the production of fibroblast KGF which contributes to re-epithelialization and wound closure (9, 28, 29).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human KGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human KGF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human KGF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human KGF 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 supernates, human serum and human plasma.
- 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 RD5R or Calibrator Diluent RD6-15 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 forty separate assays to assess inter-assay precision.

CELL CULTURE SUPERNATE ASSAY

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	82.4	248	1031	86.5	266	1108
Standard Deviation	3.2	8.8	55.2	4.8	11.4	62.0
CV%	3.9	3.5	5.4	5.5	4.3	5.6

SERUM/PLASMA ASSAY

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	97.9	298	1229	95.5	294	1221
Standard Deviation	3.3	8.8	42.8	7.4	15.6	63.5
CV%	3.4	3.0	3.5	7.7	5.3	5.2

B. RECOVERY

The recovery of human KGF 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=5)	102	95-105
Human serum (n=5)	93	85-100
Human EDTA plasma (n=5)	96	85-104
Human heparin plasma (n=5)	90	85-99
Human Citrate plasma (n=5)	91	86-98

C. SENSITIVITY

The minimum detectable dose (MDD) of human KGF is typically less than 15 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 *E.coli*-expressed recombinant human KGF produced at R&D Systems.

The NIBSC/WHO Reference Reagent 03/148 for KGF was evaluated in this kit. The dose response curve of the reference reagent 03/148 parallels the Valukine standard curve. To convert sample values obtained with the Valukine Human KGF kit to approximate NIBSC/WHO 03/148 units, use the equation below.

NIBSC (03/148) value (U/mL) = 0.0014 x Valukine Human KGF value (pg/mL).

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human KGF and diluted with Calibrator Diluent RD5R/Calibrator Diluent RD6-15 to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=5)	Human serum (n=5)	Human EDTA plasma (n=5)	Human heparin plasma (n=5)	Human citrate plasma (n=5)
1:2	Average % of Expected	99	100	101	103	104
	Range (%)	98-100	98-102	99-104	101-106	103-107
1:4	Average % of Expected	99	103	103	104	104
	Range (%)	97-102	101-107	102-106	101-106	100-108
1:8	Average % of Expected	96	105	104	106	108
	Range (%)	93-99	102-108	102-106	104-108	104-115
1:16	Average % of Expected	94	103	103	107	103
	Range (%)	89-97	98-106	101-107	104-116	96-108

F. SAMPLE VALUES

Human Serum/Plasma - Eighty-five serum and plasma samples from apparently healthy volunteers were evaluated for the presence of KGF in this assay. All samples measured below the lowest Human KGF Standard, 31.3 pg/mL. No medical histories were available for the donors used in this study

Cell Culture Supernates - NHDF human normal dermal fibroblasts were cultured in fibroblast basal media supplemented with 1 µg/mL of recombinant human FGF, 5 mg/mL insulin, 50 mg/mL gentamycin, 50 µg/mL amphotericin-B, and 10% heat inactivated fetal calf serum. Cells were stimulated with the agents listed in the table below. Aliquots of the cell culture supernates were removed after 2 days and assayed for levels of natural human KGF.

Stimulant	Day 2 (pg/mL)
Control	106
Human IL-1β	258
Human TNF-α	208
Human IL-6	110

G. SPECIFICITY

This assay recognizes natural and recombinant human KGF.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5R/Calibrator Diluent RD6-15 and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human KGF control were also assayed for interference. No significant cross-reactivity or interference was observed.

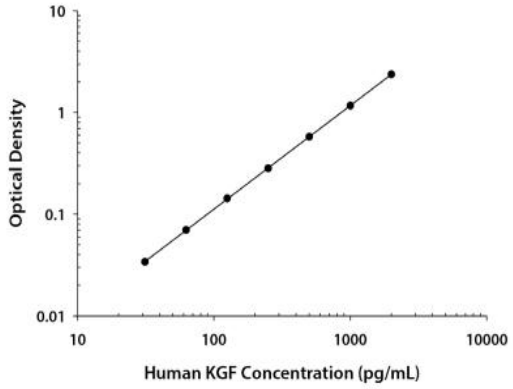
Recombinant human：	**Recombinant mouse:**
β-ECGF	IL-1α
EGF	IL-1β
Epo	IL-6
FGF-4	LIF
FGF-5	**Recombinant amphibian:**
FGF-6	TGF-β5
FGF acidic	**Natural proteins:**
FGF basic	bovine FGF acidic
HB-EGF	bovine FGF basic
HGF	human PDGF
IL-1α	porcine PDGF
IL-1β	human TGF-β1
IL-6	porcine TGF-β1
LAP (TGF-β1)	porcine TGF-β2
LIF	
PD-ECGF	
PDGF-AA	
PDGF-AB	
PDGF-BB	
VEGF	

IV. EXPERIMENT

EXAMPLE STANDARD

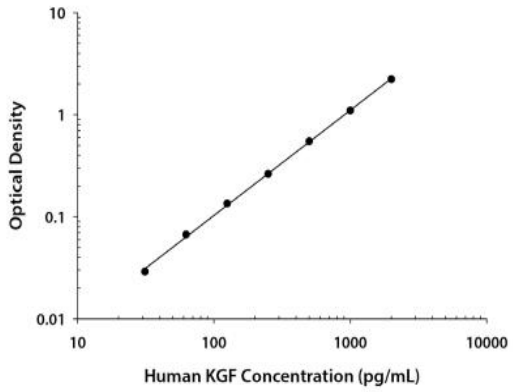
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.026 0.033	0.030	—
31.3	0.066 0.063	0.064	0.034
62.5	0.100 0.100	0.100	0.070
125	0.174 0.172	0.173	0.143
250	0.319 0.307	0.313	0.283
500	0.608 0.607	0.608	0.578
1000	1.157 1.235	1.196	1.166
2000	2.466 2.342	2.404	2.374

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.037 0.041	0.039	—
31.3	0.069 0.066	0.068	0.029
62.5	0.108 0.103	0.106	0.067
125	0.177 0.172	0.174	0.135
250	0.301 0.302	0.302	0.263
500	0.594 0.584	0.589	0.550
1000	1.159 1.119	1.139	1.100
2000	2.267 2.256	2.262	2.223

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human KGF Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human KGF	1 plate
Human KGF Conjugate	Solution of antibody against human KGF conjugated to horseradish peroxidase	1 vial
Human KGF Standard	Recombinant human KGF in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume	2 vials
Assay Diluent RD1-25	A buffered protein base	1 vial
Calibrator Diluent RD5R	A buffered protein base used to dilute standard and samples (*For cell culture supernate samples*)	1 vial
Calibrator Diluent RD6-15	A buffered protein base used to dilute standard and samples (*For serum/plasma samples*)	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution	2 vials
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="8">Opened/ Reconstituted Reagents</td><td>Wash Buffer (1×)</td><td rowspan="6">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>Assay Diluent RD1-25</td></tr>
<tr><td>Calibrator Diluent RD5R</td></tr>
<tr><td>Calibrator Diluent RD6-15</td></tr>
<tr><td>TMB Substrate</td><td rowspan="2">Use a fresh standard for each assay.
Discard after use.</td></tr>
<tr><td>Standard</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.
- Polypropylene test tubes for dilution of standards.

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 eye, hand, face, and clothing protection when using this material.

VI. PREPARATION

A. SAMPLE COLLECTION AND 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. Samples may require dilution with Calibrator Diluent RD5R.

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 × 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 RD6-15.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent RD6-15.

B. REAGENT PREPARATION

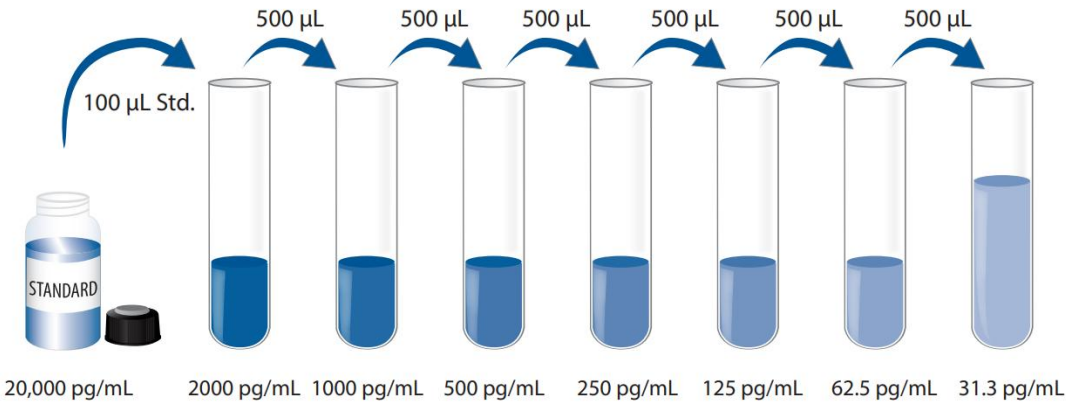
Bring all reagents to room temperature before use.

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×).

Human KGF Standard - Refer to the vial label for the reconstitution volume. * Reconstitute the Human KGF Standard with deionized or distilled water. This reconstitution produces a stock solution of 20000 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.

Use polypropylene tubes. Pipette 900 µL of the Calibrator Diluent RD5R (*for cell culture supernate samples)* or Calibrator Diluent RD6-15 (*for serum/plasma samples*) into the 2000 pg/mL tube. Pipette 500 µL of the appropriate Calibrator Diluent RD5R (*for cell culture supernate samples*) or Calibrator Diluent RD6-15 (*for serum/plasma samples)* into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent RD5R (*for cell culture supernate samples*) or Calibrator Diluent RD6-15 (*for serum/plasma samples*) serves as the zero standard (0 pg/mL).



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

1. Prepare all reagents, working standards 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 100 µL of Assay Diluent RD1-25 to each well.
4. Add 100 µL of standard and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 3 hours at room temperature.** A plate layout is provided for a record of 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 KGF Conjugate to each well. Cover with a new adhesive strip. **For Cell Culture Supernate Samples: Incubate for 1.75 hours at room temperature. For Serum/Plasma Samples: Incubate for 2 hours at room temperature.**
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. 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.

11. CALCULATION OF RESULTS

Average the duplicate readings for each standard 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 log/log 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 a log/log graph. The data may be linearized by plotting the log of the human KGF concentrations versus the log of the O.D. on a linear scale 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.

VIII. REFERENCES

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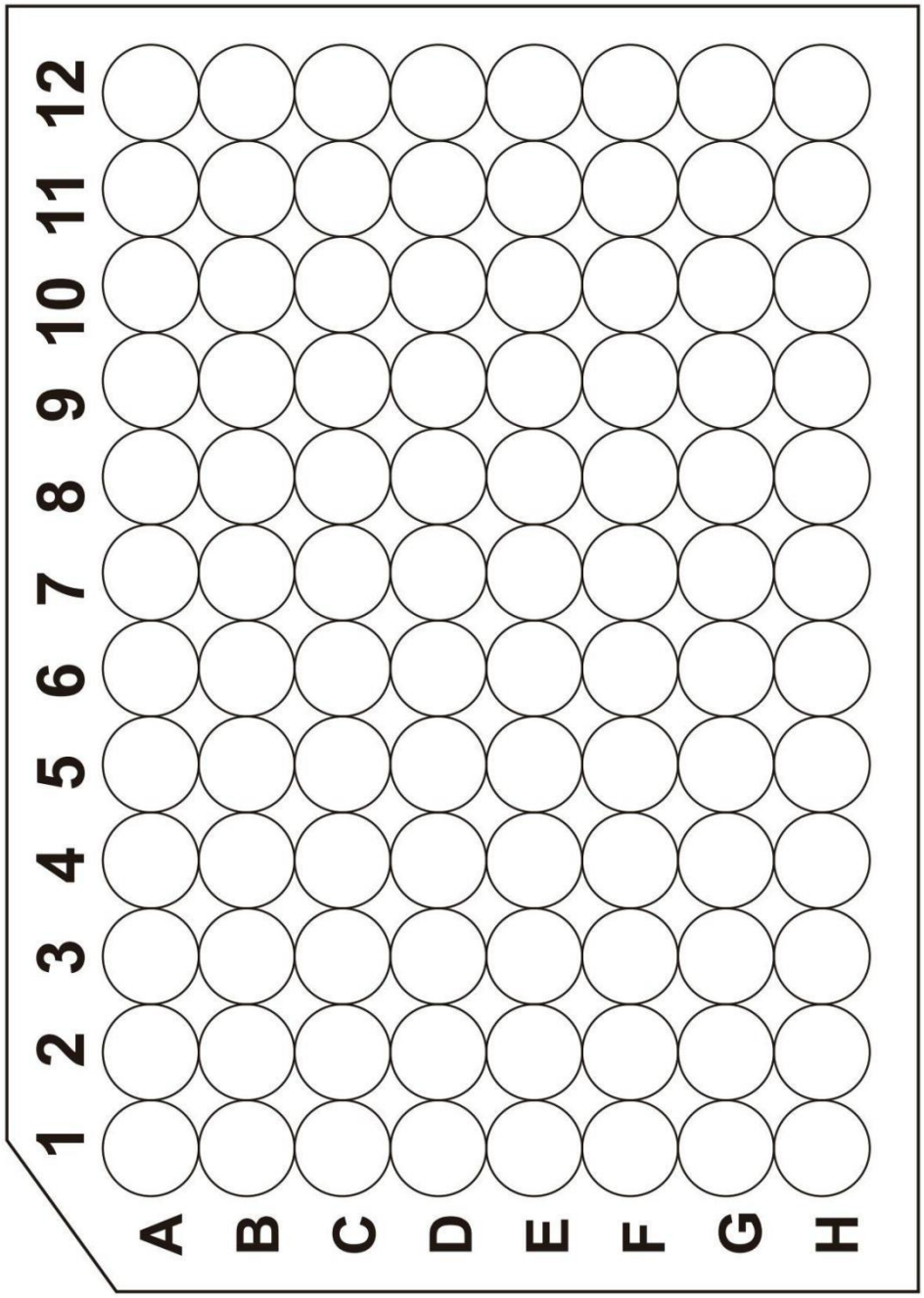
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 KGF/FGF-7 Valukine™ ELISA 试剂盒

目录号：VAL209

适用于定量检测天然和重组人角质细胞生长因子 (KGF) 的浓度

科研专用，不可用于临床诊断

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版本号 202410.1

目录

I. 背景

KGF 是一种单链、肝素结合型分子量 28 kDa 的糖蛋白。最初是从人胚胎肺成纤维细胞生长的培养基中分离出来的（1, 2）。它也被称为 FGF-7，是快速发展的成纤维细胞生长因子家族的一员，目前包括 14 个成员(3-6)。成熟的 KGF 有 163 个氨基酸 (aa) 残基，含有五个半胱氨酸。这五个半胱氨酸不是活性所必需的，但有助于肝素结合(2, 7)。在 FGF 家族中，KGF 与 FGF-2 有 29%的氨基酸序列相同，与 FGF-3 有 38%的氨基酸序列相同(8)。据报道，表达 KGF 的细胞包括成纤维细胞（1, 9）、胚胎间充质细胞（10-12）和平滑肌细胞（13）。

KGF R 是细菌表达激酶基因产物的限制表达剪接变体，是一种具有酪氨酸激酶活性的细胞表面受体，也被称为 FGF R2(14-16)。FGF R2 是一种全长、未剪接（或标准），分子量为 135 kDa 的 I 型跨膜糖蛋白，其细胞外结构域含有三个 Ig 样结构，还有一个域间序列的肝素结合基团连接 N 端（D1）和中间（D2）Ig-结构（16）。这种标准受体形式在结缔组织细胞中普遍表达（17），并能与 FGF-1、FGF-2 和 FGF-4 具有高亲和力（Kd ~ 100 pM）。FGF-5 和 FGF-9 也能结合，但亲和力较低（Kd ~2 nM）（16、18-20）。KGF R 剪接变体与标准受体的区别仅在于第三个（或膜近端）Ig 样结构域（D3）中的一个 49 aa 残基序列（15, 21）。虽然这种变化对 FGF-1 的结合影响不大（Kd = 600 pM），但它的存在会降低与 FGF-2 的结合（Kd = 3 nM）（15），但能与 KGF 结合（Kd = 200 pM）。与 KGF 一样，表达 KGF R 的细胞数量很少，且仅限于上皮细胞类型，如角质形成细胞（22）、过渡上皮细胞（但不是伞状细胞）（23）、胃柱状上皮细胞（24）、胚胎肺上皮细胞（10）、乳腺上皮细胞（25）和肝细胞（26）。除 KGF R 外，KGF 还能与 HSPG 结合。一般来说，HSPGs 在调节 FGFs 生物活性方面所起的作用尚不清楚，尽管它们被认为有助于 FGFs 与其高亲和力酪氨酸激酶受体结合。有人认为，HSPGs 可能会将两个 FGF 分子紧紧靠在一起，从而使两个单独的 FGF-FGF R 复合物发生二聚化，或者形成一个 FGF-HSPG 复合物，该复合物可主动结合两个独立的 FGF 受体（16）。然而，特别是对于 KGF 而言，HSPGs 对 KGF 的活性没有影响或有抑制作用。因此，关于硫酸肝素对 KGF 的结合和生物活性的重要性还没有达成共识（27）。

在功能上，KGF 被认为是许多不同类型上皮细胞的旁分泌效应因子（1, 10, 11, 26, 27）。它由真皮层或固有层成纤维细胞合成，仅局部作用于上皮表层。它除了能诱导细胞增殖（27），还能促进上皮细胞分化（12）。在伤口愈合过程中，促炎分子的存在对 KGF 的再上皮化作用起到了补充作用。细胞因子，如 IL-1（α 和 β）和 IL-6，不仅能激活局部结缔组织细胞，导致异物清除和组织重塑，而且也会刺激成纤维细胞 KGF 的产生，从而促进伤口的再上皮化和闭合（9, 28, 29）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人KGF抗体包被于微孔板上，样品和标准品中的人KGF会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶标记的抗人KGF检测抗体进行孵育。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，人血清样本和人血浆样本；
- 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，应将样本用标准品稀释液RD5R或标准品稀释液RD6-15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，在不同板间分别检测40次，以确定板间精确度。

细胞培养上清试验

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	82.4	248	1031	86.5	266	1108
标准差	3.2	8.8	55.2	4.8	11.4	62.0
CV%	3.9	3.5	5.4	5.5	4.3	5.6

血清/血浆试验

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	97.9	298	1229	95.5	294	1221
标准差	3.3	8.8	42.8	7.4	15.6	63.5
CV%	3.4	3.0	3.5	7.7	5.3	5.2

B. 回收率

不同类型样本中掺入检测范围内不同水平的人KGF，测定其回收率。

样本类型	平均回收率 (%)	范围 (%)
细胞培养基 (n=5)	102	95-105
人血清样本 (n=5)	93	85-100
人 EDTA 血浆样本 (n=5)	96	85-104
人肝素血浆样本 (n=5)	90	85-99
人枸橼酸钠血浆样本 (n=5)	91	86-98

C. 灵敏度

人KGF的最低可检测剂量（MDD）一般小于15 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

该免疫测定法以R&D Systems生产的高纯度的大肠杆菌表达的重组人KGF校正。

使用NIBSC/WHO标准试剂03/148检测KGF。标准试剂03/148的剂量响应曲线与Valukine标准曲线平行。要将人KGF Valukine试剂盒获得的样品值转换为近似NIBSC/WHO 03/148单位，请使用以下公式。

NIBSC (03/148) 检测值 (U/mL) = 0.0014 x 人KGF Valukine 检测值 (pg/mL) 。

E. 线性

不同的样本中含有或掺入高浓度的人KGF，然后用标准品稀释液RD5R/标准品稀释液RD6-15将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养基 (n=5)	人血清 (n=5)	人EDTA血浆 (n=5)	人肝素血浆 (n=5)	人枸橼酸钠血浆 (n=5)
1:2	平均值/期待值 (%)	99	100	101	103	104
	范围 (%)	98-100	98-102	99-104	101-106	103-107
1:4	平均值/期待值 (%)	99	103	103	104	104
	范围 (%)	97-102	101-107	102-106	101-106	100-108
1:8	平均值/期待值 (%)	96	105	104	106	108
	范围 (%)	93-99	102-108	102-106	104-108	104-115
1:16	平均值/期待值 (%)	94	103	103	107	103
	范围 (%)	89-97	98-106	101-107	104-116	96-108

F. 样本预值

人血清/血浆 - 使用此试剂盒评估 85 份表面健康志愿者血清和血浆样本中 KGF 的存在。所有样本检测值均低于人 KGF 最低标准，31.3 pg/mL。本研究中使用的供体没有病史。

细胞培养上清 - NHDF人正常真皮成纤维细胞培养在含1 μg/mL重组人FGF、5 mg/mL胰岛素、50 mg/mL庆大霉素、50 μg/mL两性霉素- B和10%热灭活胎牛血清的成纤维细胞基础培养基中。用下表中列出的药物刺激细胞。培养2天，取出等量的细胞培养上清，检测人KGF的水平。

刺激类型	2天 (pg/mL)
对照组	106
人 IL-1β	258
人 TNF-α	208
人 IL-6	110

G. 特异性

此ELISA法可检测天然及重组人KGF。

将以下因子用标准品稀释液RD5R/标准品稀释液RD6-15配制成50 ng/mL的浓度来检测与人KGF的交叉反应。将50 ng/mL的干扰因子掺入中间范围的重组人KGF对照品中，来检测对人KGF的干扰。没有观察到明显的交叉反应或干扰。

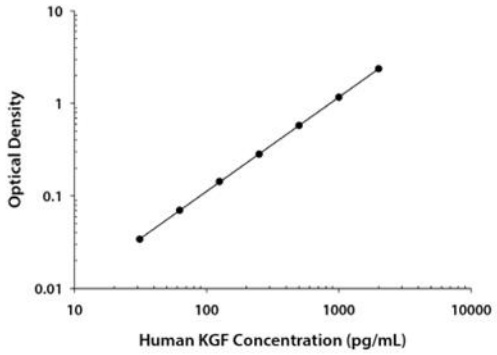
Recombinant human：	**Recombinant mouse:**
β-ECGF	IL-1α
EGF	IL-1β
Epo	IL-6
FGF-4	LIF
FGF-5	**Recombinant amphibian:**
FGF-6	TGF-β5
FGF acidic	**Natural proteins:**
FGF basic	bovine FGF acidic
HB-EGF	bovine FGF basic
HGF	human PDGF
IL-1α	porcine PDGF
IL-1β	human TGF-β1
IL-6	porcine TGF-β1
LAP (TGF-β1)	porcine TGF-β2
LIF	
PD-ECGF	
PDGF-AA	
PDGF-AB	
PDGF-BB	
VEGF	

IV. 实验

标准曲线实例

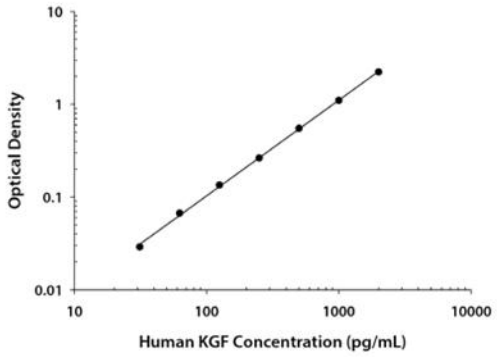
该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.026 0.033	0.030	—
31.3	0.066 0.063	0.064	0.034
62.5	0.100 0.100	0.100	0.070
125	0.174 0.172	0.173	0.143
250	0.319 0.307	0.313	0.283
500	0.608 0.607	0.608	0.578
1000	1.157 1.235	1.196	1.166
2000	2.466 2.342	2.404	2.374

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.037 0.041	0.039	—
31.3	0.069 0.066	0.068	0.029
62.5	0.108 0.103	0.106	0.067
125	0.177 0.172	0.174	0.135
250	0.301 0.302	0.302	0.263
500	0.594 0.584	0.589	0.550
1000	1.159 1.119	1.139	1.100
2000	2.267 2.256	2.262	2.223

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human KGF Microplate	包被抗人KGF抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Human KGF Conjugate	酶标检测抗人KGF抗体	1瓶
Human KGF Standard	人KGF标准品（冻干），参考瓶身标签进行重溶	2瓶
Assay Diluent RD1-25	检测液	1瓶
Calibrator Diluent RD5R	标准品稀释液用于稀释标准品和样品（*用于细胞培养上清样本*）	1瓶
Calibrator Diluent RD6-15	标准品稀释液用于稀释标准品和样品（*用于血清/血浆样本*）	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	2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>洗涤液（1×）</td><td rowspan="6">2-8°C储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>检测液RD1-25</td></tr>
<tr><td>标准品稀释液RD5R</td></tr>
<tr><td>标准品稀释液RD6-15</td></tr>
<tr><td>TMB底物溶液</td><td>每次检测都使用新的标准品。使用后丢弃</td></tr>
<tr><td>标准品</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 °C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
- 标准品稀释用聚丙烯管

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。

VI. 实验前准备

A. 样品收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

细胞培养上清液 - 通过离心去除颗粒物，立即或等分进行检测，并将样品储存在≤ -20°C的温度下，避免反复冻融。样品可能需要用标准品稀释液RD5R稀释。

血清 - 使用血清分离管(SST)，让样本在室温下凝固 30 分钟，然后在 1000 × g 的离心力下离心 15 分钟。分离血清并立即进行检测，或分装并储存在 ≤ -20 °C。避免反复冻融循环。样本可能需要用标准品稀释液 RD6-15 进行稀释。

血浆 - 使用EDTA、肝素或枸橼酸钠作为抗凝剂收集血浆。然后1000 × g离心15分钟。需在30分钟内收集血浆样本之后立即检测，或分装并储存在 ≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液 RD6-15 稀释。

B. 检测前准备工作

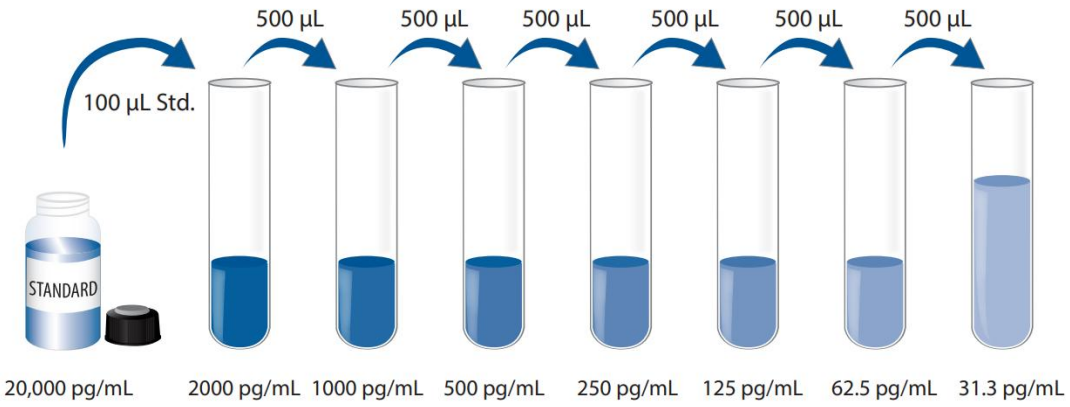
使用前请将所有试剂放置于室温。

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×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

人KGF标准品：重溶体积请参考瓶身标签*，用去离子水或蒸馏水重溶人KGF标准品，得到浓度为20000 pg/mL标准品储备母液。轻轻震摇至少15分钟，其充分溶解。

*如有疑问，请咨询我们的技术支持。

使用聚丙烯管。将900 µL标准品稀释液RD5R（*用于细胞培养上清液样本*）或标准品稀释液RD6-15（*用于血清/血浆样本*）移入2000 pg/mL管中。将500 µL标准品稀释液RD5R（*用于细胞培养上清液样本*）或标准品稀释液RD6-15（*用于血清/血浆样本*）移入剩余管中。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。2000 pg/mL作标准曲线最高点，标准品稀释液RD5R（*用于细胞培养上清液样本*）或标准品稀释液RD6-15（*用于血清/血浆样本*）移入可用作标准曲线零点（0 pg/mL）。



C. 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；

VII.操作步骤

使用前，将所有其他试剂和样品带至室温。建议对所有标准品和样品进行复孔检测。

1. 按照上一节的说明，准备好所有需要的试剂，标准品和样本。
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. 向每个孔中加入100 µL检测液RD1-25。
4. 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，**在室温下孵育3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。
5. 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。
6. 在每个微孔内加入200 µL人KGF酶标检测抗体。用封板膜封住反应孔，**针对于细胞培养上清样本：在室温下孵育1.75小时；针对于血清/血浆样本：在室温下孵育2小时**。
7. 重复第5步洗板操作。
8. 在每个微孔内加入200 µL TMB底物溶液，**室温孵育30分钟。注意避光**。
9. 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。
10. 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。
11. **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log/log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log/log图上的点绘制最佳拟合曲线。数据可以通过绘制人KGF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。

    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。

VIII. 参考文献

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96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置