PRODUCT INFORMATION & MANUAL

LR3 IGF-1 Valukine™ ELISA

VAL172

For the quantitative determination of LR3 IGF-1
in cell culture supernatants

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

Version202210.1

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I. BACKGROUND

Insulin-like Growth Factor 1 (IGF-1 or IGF-I), also known as Somatomedin C, is the dominant effector of Growth Hormone (GH) and is structurally homologous to Proinsulin. Human IGF-1 is synthesized as two precursor isoforms with N- and alternative C‑terminal propeptides (1). These isoforms are differentially expressed by various tissues (1). The 7.6 kDa mature IGF‑1 is identical between isoforms and is generated by proteolytic removal of the N- and C-terminal regions. Mature human IGF-1 shares 94% and 96% amino acid (aa) sequence identity with the mouse and rat orthologs, respectively (2). GH stimulates the production of IGF-1 in most tissues (3). Hepatocytes produce circulating IGF-1, while local IGF-1 is produced by many other tissues in which it has paracrine effects (1). IGF-1 induces the proliferation, migration, and differentiation of a wide variety of cell types during development and postnatally (4, 5). IGF-1 regulates glucose, fatty acid, and protein metabolism, steroid hormone activity, and cartilage and bone metabolism (6-11). It plays an important role in muscle regeneration and tumor progression (1, 12, 13). IGF-1 binds IGF1R, IGF2R, and the Insulin Receptor, although its effects are mediated primarily by IGF1R (14). IGF-1 also binds with strong affinity to IGF binding proteins (IGFBPs), which regulate the availability and biological activities of IGF-1 (15, 16).

Long R3 IGF-1 (LR3 IGF-1) is a 9.2 kDa synthetic analog of IGF-1 that is generated by modifying the aa sequence for mature human IGF-1. These modifications include the substitution of an Arg for Glu at position 3 of the mature IGF-1 sequence and the addition of a thirteen aa N-terminal extension, which is derived from methionyl porcine Growth Hormone (17). These aa changes generate a protein that is still capable of binding to IGF-1 and Insulin receptors, but shows considerably lower affinity binding to IGFBPs compared to wild-type IGF-1 (17, 18). As a result, LR3 IGF-1 has an increased half-life and displays increased biological potency compared to IGF-1 (17-22).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for LR3 IGF-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any LR3 IGF-1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for LR3 IGF-1 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 solution is added to the wells and color develops in proportion to the amount of LR3 IGF-1 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 supernatants.
- 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 (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 (ng/mL)	2.69	6.47	19.93	2.71	6.46	19.80
Standard Deviation	0.08	0.07	0.82	0.08	0.10	0.79
CV%	3.0	1.1	4.1	3.0	1.5	4.0

B. RECOVERY

The recovery of LR3 IGF-1 spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 92.6-119.6% with an average of 107.7%.

C. SENSITIVITY

The minimum detectable dose (MDD) of LR3 IGF-1 is typically less than 0.11 ng/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 LR3 IGF-1 produced at Prime Gene.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of LR3 IGF-1 and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	99.6	95.5-102.5
1:4	99.8	94.7-106.4
1:8	100.4	94.7-106.4
1:16	107.6	100.0-118.2

F. SPECIFICITY

The following factors were tested in the assay at 20 ng/mL and no cross-reactivity was identified.

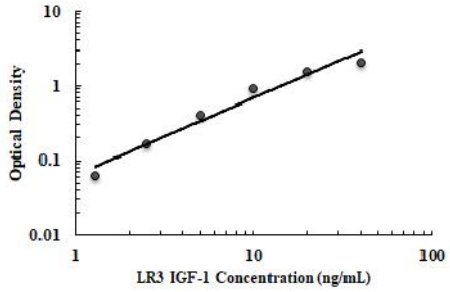
Recombinant human	Other recombinants
IGF-2	mouse IGF-1
IGF-BP3	rat IGF-1
IGF-BP5	
IGF-BP7	
Insulin	

The kit showed high cross-reactivity against human IGF-1. If the sample contains human IGF-1, it will affect the test result.

IV. EXPERIMENT

EXAMPLE STANDARD

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



ng/mL	OD	Average	Corrected
0	0.065 0.074	0.070	-
1.3	0.130 0.138	0.134	0.064
2.5	0.229 0.240	0.235	0.165
5	0.464 0.502	0.483	0.413
10	0.961 1.000	0.981	0.911
20	1.558 1.693	1.626	1.556
40	2.132 2.168	2.150	2.080

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
LR3 IGF-1 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse antibody against LR3 IGF-1.	1 plate
LR3 IGF-1 Standard	Recombinant LR3 IGF-1 in a buffered base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
LR3 IGF-1 Detection Antibody	Biotinylated LR3 IGF-1 antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent (1×)	Buffered diluent used to dilute standard and samples.	1 vial
Reagent Diluent (1×)	Buffered protein base used to dilute Detection Antibody and Streptavidin-HRP.	1 vial
Streptavidin-HRP C (100×)	100× Streptavidin conjugated to horseradish peroxidase.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	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="7">Opened/ Reconstituted Reagents</td><td>Streptavidin-HRP C</td><td rowspan="4">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</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 (1×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Detection Antibody (1×) and Streptavidin-HRP (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Calibrator Diluent (1×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Prepare fresh Standard 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.

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.
- 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 require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

Cell culture supernates samples require a 4-fold dilution. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent (1×).

C. REAGENT PREPARATION

Note: *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×).

Detection Antibody- Centrifuge briefly before opening. Reconstitution Volume refer to vial label with Reagent Diluent (1×). Aliquot and store if needed. Allow the detection antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Dilute stock solution in Reagent Diluent (1×) to the working concentration of 30 ng/mL. Prepare at least 15 minutes prior to use.

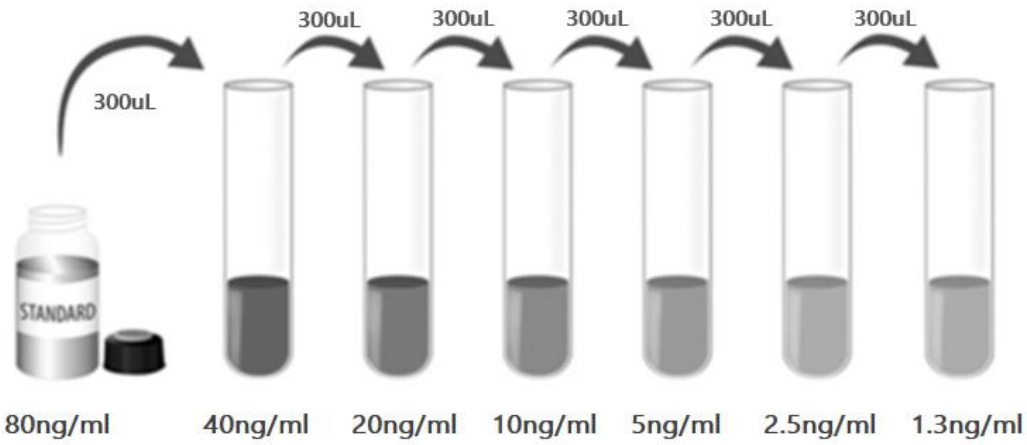
Streptavidin-HRP C (1×) - Centrifuge briefly before opening. Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

LR3 IGF-1 Standard - Centrifuge briefly before opening. Refer to the vial label for the reconstitution volume*. This reconstitution produces a stock solution of 80 ng/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 the appropriate Calibrator Diluent (1×) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 40 ng/mL standard serves as the high standard. The Calibrator

Diluent (1×) serves as the zero standard (0 ng/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

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

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, or prepared sample per well. Cover with the adhesive strip provided. Incubate for **3 hours at 2-8 °C**. 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 Solution (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 diluted in Reagent Diluent, to each well. Cover with a new adhesive strip and incubate **1 hour at 2-8 °C**.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working dilution of Streptavidin-HRP C to each well. Cover the plate and incubate for **30 minutes at 2-8 °C**. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of TMB Substrate to each well. Incubate for **20 minutes at room temperature**. Avoid placing the plate in direct 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, 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 LR3 IGF-1 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.

VIII. REFERENCES

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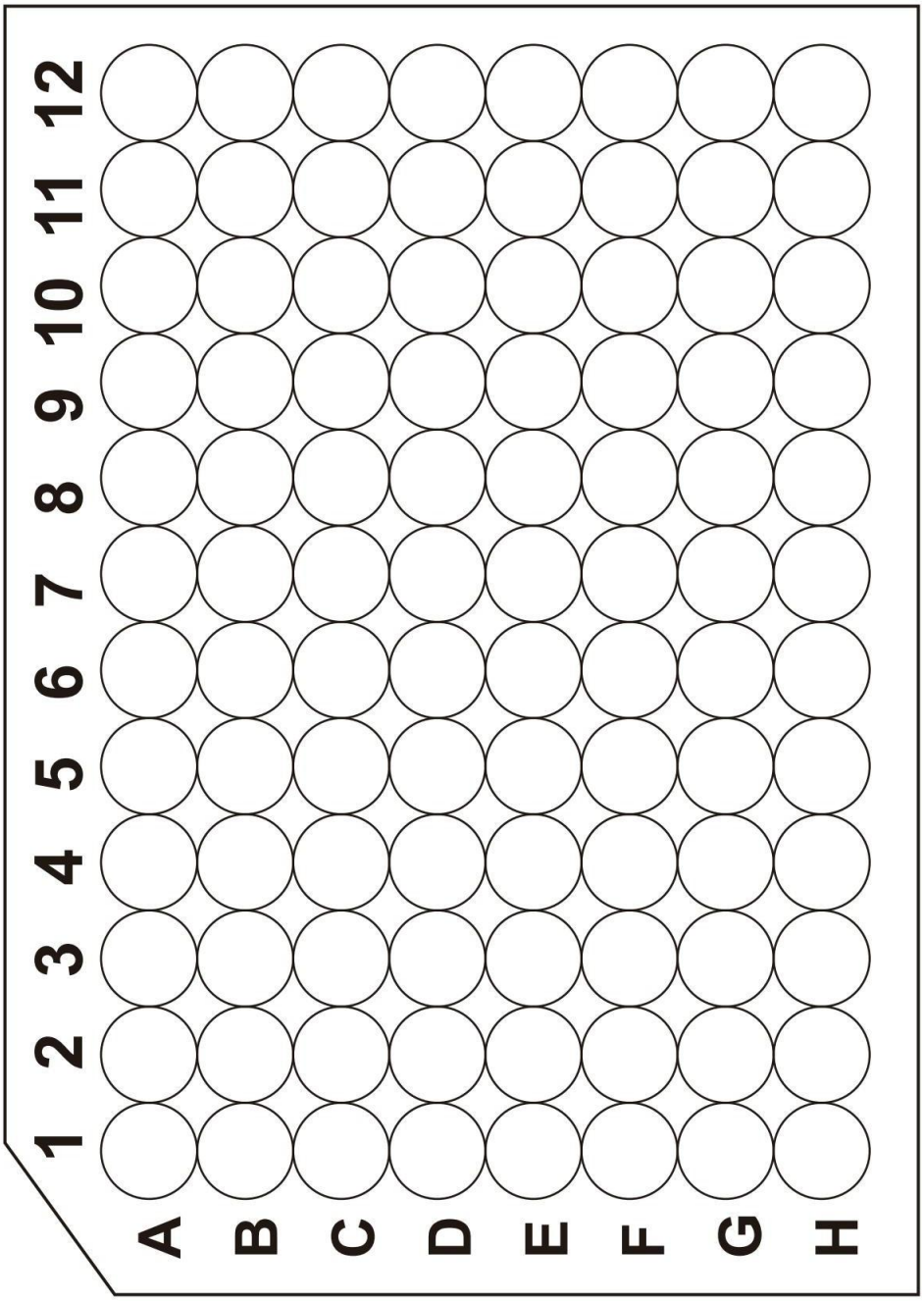
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

LR3 IGF-1 Valukine™ ELISA 试剂盒

目录号：VAL172

适用于定量检测细胞培养上清 LR3 IGF-1 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

目录

I. 背景

胰岛素样生长因子1（IGF-1或IGF-I），也称为促生长因子C，是生长激素（GH）的主要效应因子，在结构上与胰岛素原同源。人类IGF-1被合成为两种前体异构体，具有N-和可替代的C-端前肽（1）。这些亚型在不同组织中有表达差异（1）。7.6 kDa的成熟IGF-1异构体之间是相同的，并且是通过蛋白水解去除N端和C端区域产生的。成熟的人IGF-1与小鼠和大鼠的氨基酸(aa)序列分别具有94%和96%的同源性（2）。生长激素刺激大多数组织中IGF-1的产生（3）。肝细胞产生循环IGF-1，而局部IGF-1由许多其他组织产生，在这些组织中，IGF-1具有旁分泌作用（1）。IGF-1在发育过程中和出生后诱导多种细胞类型的增殖、迁移和分化（4, 5）。IGF-1调节葡萄糖、脂肪酸和蛋白质代谢、类固醇激素活性以及软骨和骨代谢（6-11）。它在肌肉再生和肿瘤进程中起着重要作用（1, 12, 13）。IGF-1结合IGF1R、IGF2R和胰岛素受体，尽管其作用主要由IGF1R介导（14）。IGF-1还与IGF结合蛋白（IGFBPs）具有很强的亲和力，IGFBPs调节IGF-1的有效性和生物活性（15, 16）。

LR3 IGF-1是一种9.2 kDa的合成的IGF-1类似物，通过修改成熟人IGF-1的aa序列产生。这些修改包括在成熟IGF-1序列的第3位用Arg替换Glu，以及添加13个aa的 N末端延伸，该延伸源于甲硫酰的猪生长激素（17）。这些aa变化产生的蛋白仍然能够与IGF-1和胰岛素受体结合，但与野生型IGF-1相比，与IGFBPs结合的亲和力显著降低（17,18）。因此，与IGF-1相比，LR3 IGF-1的半衰期延长，生物效力增强（17-22）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗LR3 IGF-1抗体包被于微孔板上，标准品和样品中的LR3 IGF-1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LR3 IGF-1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；
- 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（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、试剂盒的储存等。

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 (ng/mL)	2.69	6.47	19.93	2.71	6.46	19.80
标准差	0.08	0.07	0.82	0.08	0.10	0.79
CV%	3.0	1.1	4.1	3.0	1.5	4.0

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LR3 IGF-1，测定其回收率。回收率范围在92.6-119.6%，平均回收率在107.7%。

C. 灵敏度

LR3 IGF-1的最低可测剂量（MDD）一般小于0.11 ng/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Prime Gene生产的*E. Coli*表达的高纯度重组LR3 IGF-1蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的LR3 IGF-1，然后用标准品稀释液（1×）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	99.6%	95.5-102.5%
1:4	99.8%	94.7-106.4%
1:8	100.4%	94.7-106.4%
1:16	107.6%	100.0-118.2%

F. 特异性

将以下因子配制成20 ng/mL的浓度来检测没有观察到明显的交叉反应。

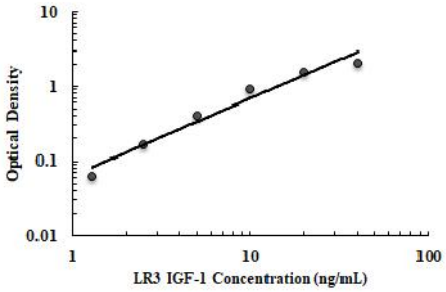
Recombinant human	Other recombinants
IGF-2	mouse IGF-1
IGF-BP3	rat IGF-1
IGF-BP5	
IGF-BP7	
Insulin	

该试剂盒对人IGF-1具有较高的交叉反应。如果样品中含有人IGF-1，则会影响检测结果。

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。



ng/mL	OD	Average	Corrected
0	0.065 0.074	0.070	-
1.3	0.130 0.138	0.134	0.064
2.5	0.229 0.240	0.235	0.165
5	0.464 0.502	0.483	0.413
10	0.961 1.000	0.981	0.911
20	1.558 1.693	1.626	1.556
40	2.132 2.168	2.150	2.080

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
LR3 IGF-1 Microplate	包被 LR3 IGF-1 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
LR3 IGF-1 Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
LR3 IGF-1 Detection Antibody	生物素化的 LR3 IGF-1 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Calibrator Diluent (1×)	标准品稀释液（1×）用于稀释标准品和样本	1 瓶
Reagent Diluent (1×)	试剂稀释液（1×）用于稀释检测抗体和链霉亲和素标记的 HRP	1 瓶
Streptavidin-HRP C (100×)	100×浓缩的链霉亲和素标记的 HRP	1 瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1 瓶
TMB Substrate	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 C</td><td rowspan="4">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（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（1×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的检测抗体工作液和链霉亲和素-HRP 工作液。</td></tr>
<tr><td>标准品稀释液（1×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的标准品</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℃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

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。
- 为防止试剂盒在检测过程中产生污染，请采取防护措施，佩戴手套和口罩等。

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。样本需要用标准品稀释液（1×）稀释。

B. 样本准备工作

细胞上清样本需要用标准品稀释液（1×）4倍稀释后进行检测，例如：50 µL样本+150 µL标准品稀释液（1×）。

C. 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浓缩洗涤液用蒸馏水或去离子水稀释配制成500mL工作浓度的洗涤液（1×）。

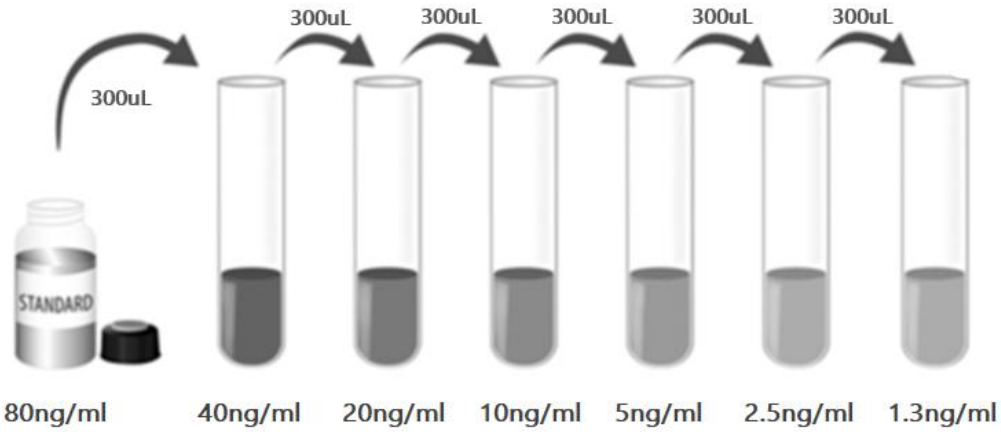
检测抗体：开盖前请瞬时离心。参考检测抗体瓶标签指示，用试剂稀释液（1×）将冻干粉进行重溶，轻轻震摇至少15分钟，使其充分溶解。再用试剂稀释液（1×）稀释至工作浓度30 ng/mL，至少在使用前15分钟准备。

链霉亲和素- HRP C（1×）：开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素- HRP C（100×）稀释至工作浓度链霉亲和素- HRP C（1×）。

LR3 IGF-1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，得到浓度为80 ng/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标准品稀释液（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。40 ng/mL管作标准曲线最高点，标准品稀释液（1×）可用作标准品零点（0 ng/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；
- 混合后的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

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、实验样本加入相应孔中，每孔100 µL。用封板膜封住反应孔，2-8 °C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；（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配制好的检测抗体工作液。用封板膜封住反应孔，2-8 °C孵育1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 C工作液。用封板膜封住反应孔，2-8 °C孵育30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 ，室温孵育20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。孔内溶液颜色会从蓝色变为黄色；
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。如果没有使用双波长校正，结果准确度可能会受影响；
12. 计算结果：将每个标准品和样品的校正吸光度值（OD450-OD540/OD570），复孔读数取平均值，然后减去平均零标准品OD值。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。另一种方法是，可以通过绘制标准品浓度做对数与相应OD值对数生成曲线，并通过回归分析确定最佳拟合线。通过样本的OD值，可从标准曲线上得到样本中LR3 IGF-1的浓度。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