PRODUCT INFORMATION & MANUAL

Mouse IGF-1 Valukine™ ELISA

VAL623

For the quantitative determination of natural and recombinant mouse IGF-1 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

Version202302.1

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I. BACKGROUND

Insulin-like Growth Factor 1 (IGF-1), also known as somatomedin C, is a member of the insulin superfamily (1, 2). It was originally discovered as a mediator of growth hormone actions on somatic cell growth, but has also been shown to be an important regulator of cell metabolism, differentiation and survival. IGF-1 is synthesized as a preproprotein that is proteolytically cleaved to generate the mature protein linked by three disulfide bonds. Mature IGF-1 is highly conserved among large mammals, with 100% sequence identity between the human, bovine, porcine, equine, and canine proteins (1).

Mature mouse IGF-1 is a non-glycosylated, 70 amino acid (aa) secreted polypeptide that is derived from either a 153 aa or a 159 aa preproprotein (3). It shares 99% and 94% aa sequence identity with rat and human IGF-1, respectively. IGF-1 is synthesized in the liver and other tissues. It is found in blood and other body fluids as a complex with specific high affinity IGF binding proteins (IGFBP-1 to -6) (4-6). The IGFBPs are expressed in specific patterns during development. They are modulators of IGF actions, which control IGF bioavailability to specific cell-surface receptors. Their functions are further regulated by IGFBP proteases, which proteolytically cleave the IGFBPs to lower the affinity with which they bind IGFs and increase IGF bioavailability. Some IGFBPs also have IGF-Independent effects on cell functions. IGF-Independent circulates primarily as a ternary complex with IGFBP-3 or IGFBP-5 and the acid-labile subunit (ALS). Some IGF-1 is also present in binary complexes with other IGFBPs. Whereas the ternary complexes are generally restricted to the vasculature, the binary complexes freely enter the tissues (4-6).

IGF-1 actions are mediated by two ubiquitously expressed receptor tyrosine kinases: IGF-I R and Insulin R/CD220. IGF-I R and Insulin R are disulfide-linked heterotetrameric complexes that consist of two alpha and two beta subunits. For both of these receptors, the prepro proteins are cleaved to produce extracellular alpha subunits which contain a cysteine-rich region and ligand-binding fibronectin type III (FN-III) domains, and beta subunits which contain an extracellular FN-III domain, transmembrane, and cytoplasmic tyrosine kinase domains. A hybrid complex containing one IGF-I R and one Insulin R also serves as a functional high affinity receptor for IGF-1. IGF-I R-Insulin R hybrids respond primarily to IGF-1, potentially downregulating the cellular response to Insulin. IGF signaling is also modulated by IGF binding proteins and the scavenger receptor, IGF-II R (7-10).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IGF-1 has been pre-coated onto a microplate. Standards, samples and a biotinylated detection antibody specific for mouse IGF-1 are pipetted into the wells and any mouse IGF-1 present is bound by the immobilized antibody. After washing away any unbound substances, streptavidin-HRP are pipetted into the wells. Following a wash to remove any unbound reagent, TMB substrate is added to the wells and color develops in proportion to the amount of mouse 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 supernate, mouse serum and 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 Reagent Diluent (1x) 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)	918.9	230.8	60.1	911.8	230.0	59.3
Standard Deviation	33.0	2.9	1.9	39.8	3.6	4.3
CV%	3.6	1.2	3.1	4.4	1.6	7.3

B. RECOVERY

The recovery of mouse IGF-1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range %**
Cell culture media (n=3)	82.9	76.3-92.7
Serum (n=4)	108.7	92.7-119.4
Plasma (n=3)	99.9	82.6-129.5

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse IGF-1 is 1.60 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 highly purified *E. coli*-expressed recombinant mouse IGF-1.

E. LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of mouse IGF-1 in various matrices and diluted with Reagent Diluent (1x) to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=3)	Serum (n=4)	Plasma (n=3)
1:2	Average % of Expected	100.0	92.6	92.6
	Range (%)	95.6-102.5	87.8-103.2	91.9-93.6
1:4	Average % of Expected	105.0	89.4	93.8
	Range (%)	98.5-108.7	86.7-90.7	89.5-96.4
1:8	Average % of Expected	115.0	96.7	103.0
	Range (%)	107.9-127.8	92.9-99.6	101.6-104.1
1:16	Average % of Expected	116.5	103.1	106.4
	Range (%)	109.2-124.6	100.6-105.5	94.5-121.3

F. SAMPLE VALUES

Serum - Four mouse serum samples were evaluated for the presence of mouse IGF-1 in this assay. All samples measured ranged from 125.7 to 741.1 ng/mL with an average of 374.3 ng/mL.

Plasma - Three mouse plasma samples were evaluated for the presence of mouse IGF-1 in this assay. All samples measured ranged from 64.8 to 393.5 ng/mL with an average of 234.6 ng/mL.

G. SPECIFICITY

This assay recognizes both natural and recombinant mouse IGF-1.

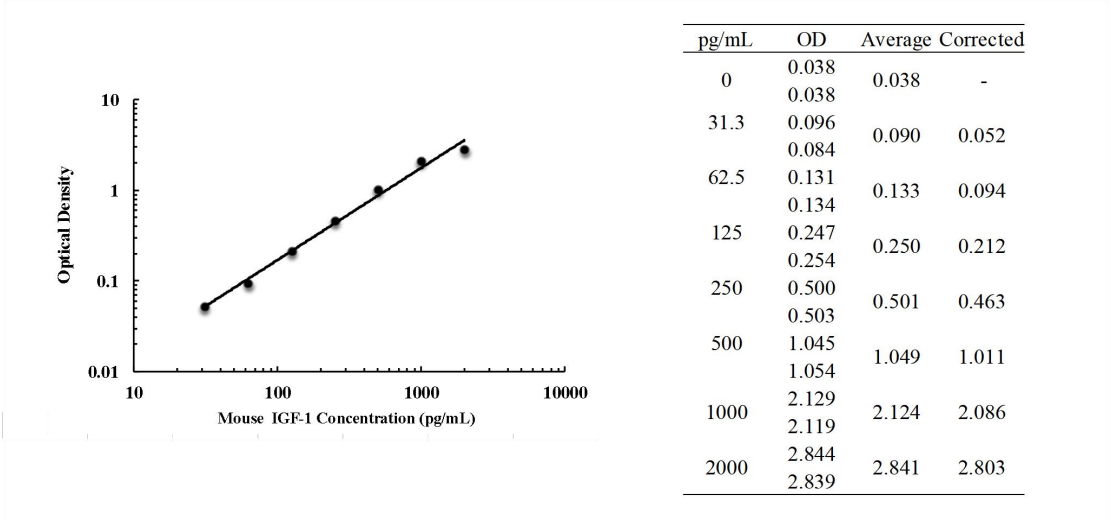
The following factors were prepared at 50 ng/mL and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rmIGF-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant Mouse	Recombinant Human
IGF-2	IGF-1
IGFBP-2	
IGFBP-5	
IGFBP-6	

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	0.038 0.038	0.038	-
31.3	0.096 0.084	0.090	0.052
62.5	0.131 0.134	0.133	0.094
125	0.247 0.254	0.250	0.212
250	0.500 0.503	0.501	0.463
500	1.045 1.054	1.049	1.011
1000	2.129 2.119	2.124	2.086
2000	2.844 2.839	2.841	2.803

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse IGF-1 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against Mouse IGF-1.	1 plate
Mouse IGF-1 Detection Antibody	Biotinylated Mouse IGF-1 antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Mouse IGF-1 Standard	Recombinant Mouse IGF-1 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Reagent Diluent Concentrate (2.5×)	A 2.5× concentrated buffered protein base used to dilute standard, samples, Detection Antibody and HRP.	2 vials
Streptavidin-HRP A (200×)	200× 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 ELISA Substrate Solution.	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Covers	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 A</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. Standard 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 (2.5×)</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>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.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernate - 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 Reagent Diluent (1×).

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ⩽ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Reagent Diluent (1×).

B. SAMPLE PREPARATION

Cell Culture Supernate requires a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Reagent Diluent (1×).

Serum and plasma samples require a 500-fold dilution. For example, add 10 µL of sample into a tube with 190 µL Reagent Diluent (1×) to prepare a 20-fold diluted sample. Mix through and then pipette 10 µL of prepared 20-fold diluted sample into a tube with 240 µL Reagent Diluent (1×) to prepare a final 500-fold diluted sample.

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.

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

Detection Antibody - Centrifuge briefly before opening. Reconstitution volume refer to vial label with Reagent Diluent (1×). Aliquot and store if needed. Dilute stock solution in Reagent Diluent (1×) to the working concentration of 0.1 ug/mL. Prepare at least 15 minutes prior to use.

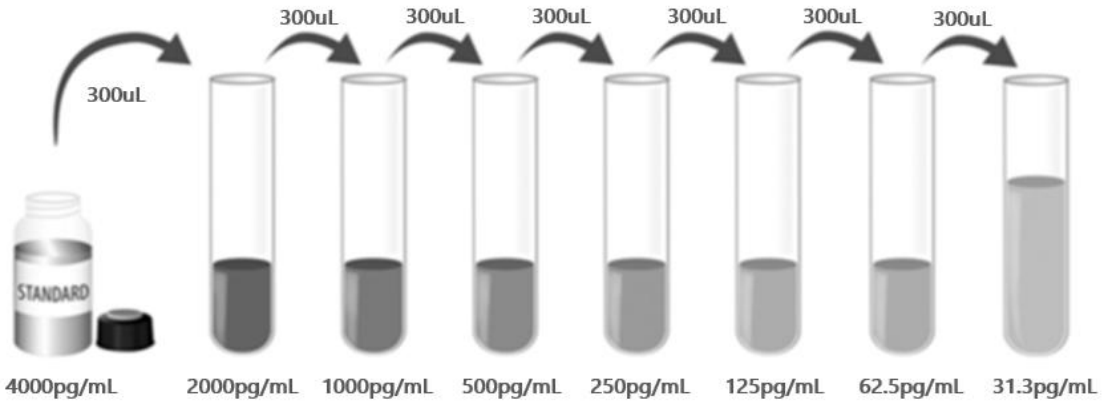
Streptavidin-HRP A (1×) - Centrifuge briefly before opening. Dilute to the working

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

Mouse IGF-1 Standard - Centrifuge briefly before opening. Refer to the vial label for reconstitution volume*. This reconstitution produces a stock solution of 4000 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×) into each tube. 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 Reagent Diluent (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

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. Add 100 µL of the Detection Antibody diluted in Reagent Diluent (1x), to each well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500 ± 50rpm.** 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 working dilution of Streptavidin-HRP A to each well. Cover the plate and incubate for 30 minutes at room temperature. **Avoid placing the plate in direct light.**
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of TMB Substrate to each well. Incubate for 20 minutes at room temperature. **Avoid placing the plate in direct light.**
8. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. 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.

10. **CALCULATION OF RESULTS：** Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse 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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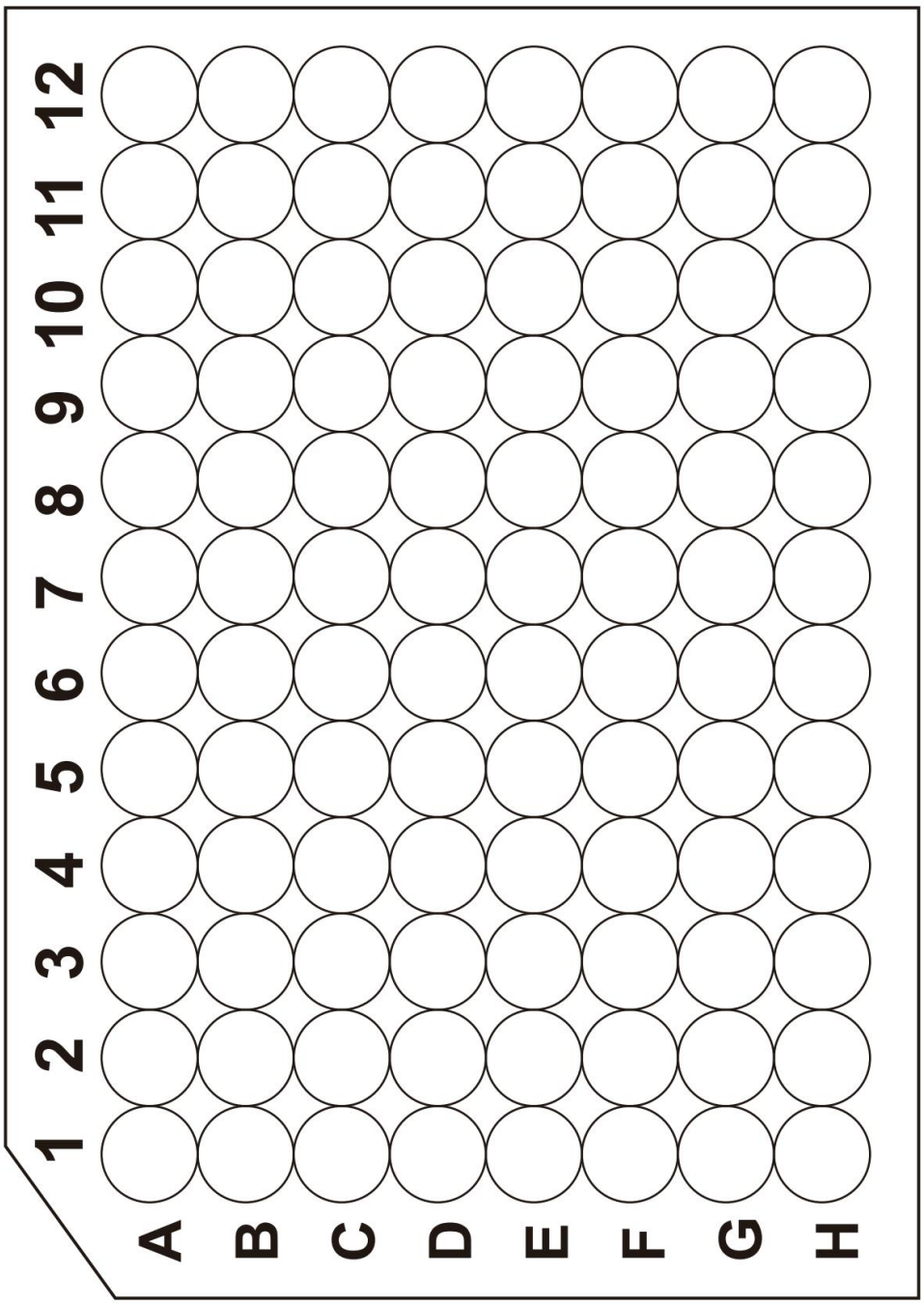
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 IGF-1 Valukine™ ELISA 试剂盒

目录号：VAL623

适用定量检测天然和重组小鼠胰岛素样生长因子 1（IGF-1）的含量

科研专用，不可用于临床诊断

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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），也称为生长激素 C，是胰岛素超家族的成员（1，2）。它最初被发现是生长激素作用于体细胞生长的介质，但也被证明是细胞代谢、分化和存活的重要调节器。IGF-1 作为一种前蛋白被合成，该前蛋白被蛋白水解裂解后生成由三个二硫键连接的成熟蛋白。成熟的 IGF-1 在大型哺乳动物中高度保守，在人、牛、猪、马和犬之间蛋白序列具有 100%的一致性（1）。

成熟小鼠 IGF-1 是一种非糖基化、70 个氨基酸（aa）分泌的多肽，来源于 153 个氨基酸或 159 个氨基酸的前蛋白（3）。它与大鼠和人 IGF-1 的氨基酸序列分别具有 99%和 94%的同源性。IGF-1 在肝脏和其他组织中合成。在血液和其他体液中发现，它是特异性高亲和力 IGF 结合蛋白（IGFBP-1 至-6）的复合体（4-6）。IGFBPs 在发育过程中以特定模式表达。它们是 IGF 作用的调节剂，控制 IGF 对特定细胞表面受体的生物利用度。它们的功能由 IGFBP 蛋白酶进一步调节，IGFBP 蛋白酶水解裂解 IGFBP 以降低它们与 IGF 结合的亲和力并提高 IGF 的生物利用度。一些 IGFBPs 对细胞功能也具有 IGF 独立效应。IGF 独立循环主要以 IGFBP-3 或 IGFBP-5 和酸不稳定亚基（ALS）的三元复合物形式进行循环。一些 IGF-1 也存在于与其他 IGFBPs 的二元复合物中。尽管三元复合物通常仅限于脉管系统，但二元复合物可自由进入组织（4-6）。

IGF-1 作用由两种广泛表达的酪氨酸激酶受体介导：IGF-I R 和 InsulinR/CD220。IGF-I R 和 Insulin R 是由两个α亚基和两个β亚基组成的二硫键连接异四聚体复合物。对于这两种受体，前体蛋白被裂解以产生胞外α亚基，其含有富含半胱氨酸的区域和配体结合的 III 型纤维连接蛋白（FN-III）结构域；以及β亚基，其含有胞外 FN-III 结构域、跨膜和胞质酪氨酸激酶结构域。含有一个 IGF-I R 和一个 Insulin R 的杂交复合物也作为 IGF-1 的功能性高亲和力受体。IGF-I R-Insulin R 复合物主要对 IGF-1 产生反应，可下调细胞对胰岛素的反应。IGF 信号也可由 IGF 结合蛋白和清道夫受体 IGF-II R 调节（7-10）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠IGF-1单抗包被于微孔板上。样品、标准品和生物素化检测抗体一起加入微孔板中，小鼠IGF-1会与固定在板上的抗体结合，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）。溶液颜色与结合的小鼠IGF-1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、小鼠血清和小鼠血浆样本；
- 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，应将样本用试剂稀释液（1×）稀释后重新检测；
- 检测结果的不同可由多种因素引起，包括实验小鼠员的操作、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，在不同板间分别检测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)	918.9	230.8	60.1	911.8	230.0	59.3
标准差	33.0	2.9	1.9	39.8	3.6	4.3
CV%	3.6	1.2	3.1	4.4	1.6	7.3

B. 回收率

在不同类型样本中掺入检测范围内不同水平的小鼠IGF-1，测定其回收率。

样本类型	平均回收率%	范围%
细胞培养上清 (n=3)	82.9	76.3-92.7
血清 (n=4)	108.7	92.7-119.4
血浆 (n=3)	99.9	82.6-129.5

C. 灵敏度

小鼠IGF-1的最低可测剂量（MDD）一般小于1.60 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试剂盒经由大肠杆菌表达的高纯度重组小鼠IGF-1蛋白所校正。

E. 线性

在不同类型样本中掺入高浓度的小鼠IGF-1，然后用试剂稀释液（1×）将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养上清 (n=3)	血清 (n=4)	血浆 (n=3)
1:2	平均值/期待值 (%)	100.0	92.6	92.6
	范围 (%)	95.6-102.5	87.8-103.2	91.9-93.6
1:4	平均值/期待值 (%)	105.0	89.4	93.8
	范围 (%)	98.5-108.7	86.7-90.7	89.5-96.4
1:8	平均值/期待值 (%)	115.0	96.7	103.0
	范围 (%)	107.9-127.8	92.9-99.6	101.6-104.1
1:16	平均值/期待值 (%)	116.5	103.1	106.4
	范围 (%)	109.2-124.6	100.6-105.5	94.5-121.3

F. 样本预值

血清样本 - 使用本试剂盒检测了4份小鼠血清样本中IGF-1的水平。4份样本的检测值在125.7-741.1 ng/mL之间，平均值为374.3 ng/mL。

血浆样本 - 使用本试剂盒检测了3份小鼠血浆样本中IGF-1的水平。3份样本的检测值在64.8-393.5 ng/mL之间，平均值为234.6 ng/mL。

G. 特异性

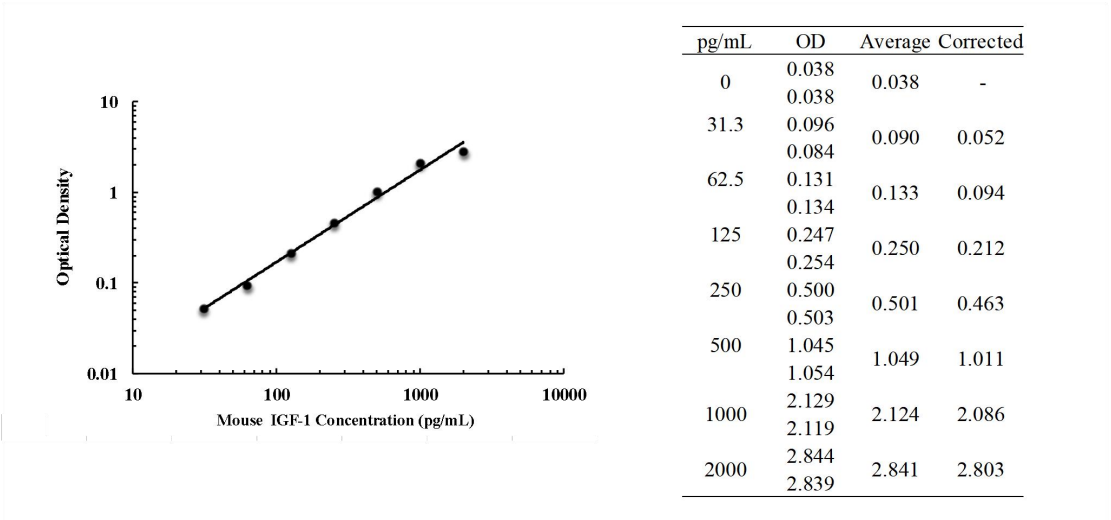
此ELISA法可检测天然及重组小鼠IGF-1蛋白。将以下因子用试剂稀释液（1×）配制成50 ng/mL的浓度来检测与小鼠IGF-1的交叉反应。将50 ng/mL的干扰因子掺入中间范围的重组小鼠IGF-1对照品中，来检测对小鼠IGF-1的干扰。没有观察到明显的交叉反应或干扰。

Recombinant Mouse	Recombinant Human
IGF-2	IGF-1
IGFBP-2	
IGFBP-5	
IGFBP-6	

IV. 实验标准

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	0.038 0.038	0.038	-
31.3	0.096 0.084	0.090	0.052
62.5	0.131 0.134	0.133	0.094
125	0.247 0.254	0.250	0.212
250	0.500 0.503	0.501	0.463
500	1.045 1.054	1.049	1.011
1000	2.129 2.119	2.124	2.086
2000	2.844 2.839	2.841	2.803

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse IGF-1 Microplate	包被抗小鼠 IGF-1 单克隆抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Mouse IGF-1 Detection Antibody	生物素化的小鼠 IGF-1 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Mouse IGF-1 Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Reagent Diluent Concentrate (2.5×)	浓缩的试剂稀释液（2.5×）用于稀释标准品、样本、检测抗体和 HRP	2 瓶
Streptavidin-HRP A (200×)	200×浓缩的链霉亲和素标记的 HRP A	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 Covers	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="7">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（2.5×）</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>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，使用时请做好眼眼睛、手、面部及衣服的防护。使用后请彻底洗手。

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。样本可能需要用试剂稀释液（1×）稀释。

血浆样本: 使用EDTA、肝素钠或枸橼酸钠作为抗凝剂收集血浆。然后1000 x g离心15分钟，需在30分钟内收集血浆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。样本可能需要用试剂稀释液（1×）稀释。

B. 样本准备工作

细胞培养上清需要用试剂稀释液（1×）2倍稀释后进行检测，即100 µL样本+100 µL试剂稀释液（1×）。

血清和血浆样本需要用试剂稀释剂（1×）500倍稀释后进行检测，例如：10 µL样本加到190 µL试剂稀释剂（1×）中，充分混匀，即20倍稀释。然后取10 µL的20倍稀释后样本加到240 µL试剂稀释剂（1×）中，充分混匀，即制备成500倍稀释的样本。

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500 mL工作浓度的洗涤液。

试剂稀释液（1×）：使用蒸馏水或去离子水稀释配制成试剂稀释液（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。再用试剂稀释液（1×）稀释至工作浓度 0.1 ug/mL，至少在使用前15 分钟准备。

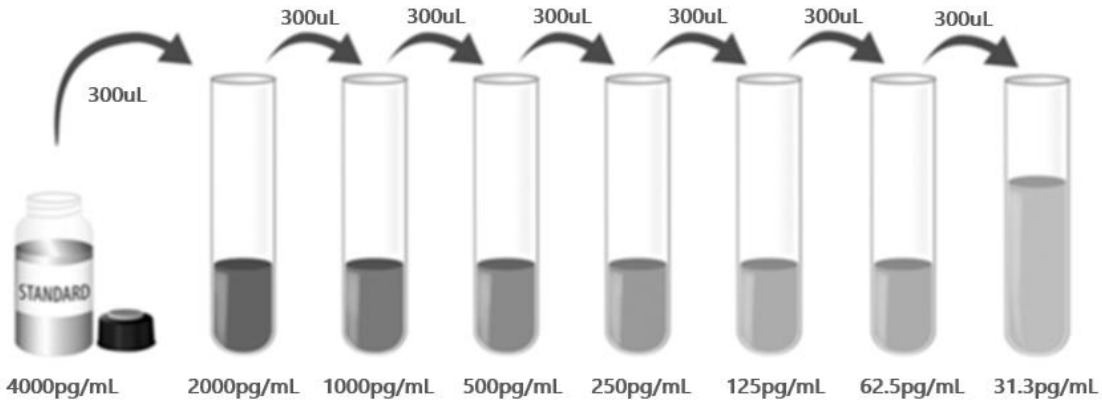
链霉亲和素- HRP A：开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素- HRP A（200×）稀释至工作浓度链霉亲和素- HRP A（1×）。

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4000

pg/mL标准品母液。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。

向各稀释管中加入300 µL试剂稀释液（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。2000 pg/mL的标准品可用作标准曲线最高点，试剂稀释液（1×）可用作标准曲线零点（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，正确使用封板胶纸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，实验样本或者质控品加入相应孔中，每孔100 µL。在每个微孔内加入100 µL配制好的检测抗体。用封板膜封住反应孔，**室温500±50rpm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，室温孵育30分钟，**注意避光**；
6. 重复第4步洗板操作；
7. 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**；
8. 在每个微孔内加入50 µL终止液，孔内溶液颜色会从蓝色变为黄色。如果溶液颜色变为绿色或者颜色变化不一致，请轻拍微孔板，使溶液混合均匀；
9. 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；
10. **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。这个过程可生成一个足够使用但不太精确的数据拟合。通过样本的OD值，可从标准曲线上得到样本中小鼠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