PRODUCT INFORMATION & MANUAL

Human IGF-2 Valukine™ ELISA

Catalog Number: VAL160

For the quantitative determination of natural and recombinant human IGF-2 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 202410.3

TABLE OF CONTENTS

I. BACKGROUND

Insulin-like Growth Factor-2 (IGF2), also known as Somatomedin-A, is a glycosylated 8 kDa Insulin family peptide hormone. It is part of a complex system of growth and metabolic regulating proteins that is particularly important during development in the nervous system, adrenal cortex, and skeletal system (1, 2). Human IGF2 is synthesized as a preproprotein that contains a 24 amino acid (aa) signal sequence, a 67 aa mature region, and an 89 aa C-terminal prosegment, commonly referred to as the E domain (3, 4). Sequential proteolytic processing generates the 18 kDa ProIGF2 followed by mature IGF2 which consists of an N-terminal B domain followed by a C domain, an A domain, and a D domain (5, 6). The B-C-A sequence is reminescent of Proinsulin, which is processed further to remove the C domain. Mature human IGF2 shares 91% and 94% aa identity with mouse and rat IGF2, respectively. Additional IGF2 related products include a splice isoform with a 56 aa N-terminal extension, "Big" IGF2 which retains 21 aa of the E domain, and Preptin (a 34 aa fragment of the E domain) which is secreted by pancreatic β-cells and facilitates both Insulin secretion and osteoblast proliferation (7-9).

IGF2 is primarily synthesized by the liver and circulates in both fetus and adult (1, 2). In the blood, mature IGF2 associates with IGFBP-1, 2, 4 or 6 and can form a ternary complex with either IGFBP-3 or IGFBP-5 plus ALS (Acid-Labile Subunit). It can additionally bind to soluble IGF2 R (which may carry 20% of total circulating IGF2) (10, 11). ProIGF2 also circulates and can form a ternary complex with IGFBP-5 and ALS (6, 11). Once dissociated from its carrier protein, mature IGF2 has mitogenic, antiapoptotic, and Insulin like activities on a wide variety of cell types. It binds and activates IGF-1 R, IGF2R, Insulin R (both A and B isoforms), and IGF-1R: Insulin R-A hybrid receptors (6, 10, 12-15).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human IGF-2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human IGF-2 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for human IGF-2 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 IGF-2 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 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 (pg/mL)	38.8	176.9	676.0	39.8	178.5	682.8
Standard Deviation	2.5	14.0	45.8	2.7	13.1	42.7
CV%	6.4	7.9	6.8	6.7	7.3	6.3

B. RECOVERY

The recovery of human IGF-2 spiked to three levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 92.0 to 104.7% with an average of 97.4%.

C. SENSITIVITY

The minimum detectable dose (MDD) of human IGF-2 is typically less than 1.24 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 IGF-2 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 IGF-2 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	100.1	93.3-111.8
1:4	105.2	93.4-121.4
1:8	109.7	93.3-124.8
1:16	100.0	91.5-115.6

F. SAMPLE VALUES

Human serum - Seven serum samples were evaluated for the presence of human IGF-2 in this assay. All samples measured ranged from 177.3 to 452.6 ng/mL with an average of 343.1 ng/mL.

G. SPECIFICITY

The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human	
IGFBP-rp1	IGF2R
IGF-1	Insulin R
IGF1R	Proinsulin

A sample containing 3.1 ng/mL of recombinant mouse IGF-2 reads as 42.3 pg/mL (1.4% cross-reactivity).

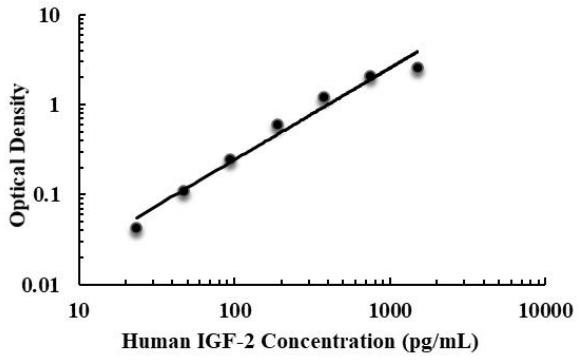
The following factors do not cross-react, but do interfere at concentrations listed below:

Recombinant Factor	**Concentration**
human IGFBP-1	> 2500 pg/mL
human IGFBP-2	> 12.5 pg/mL
human IGFBP-3	> 0.8 pg/mL
human IGFBP-4	> 125 pg/mL
human IGFBP-5	> 1563 pg/mL
human IGFBP-6	> 0.8 pg/mL

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	0.080 0.078	0.079	-
23.4	0.123 0.121	0.122	0.043
46.9	0.187 0.188	0.188	0.109
93.8	0.329 0.327	0.328	0.249
187.5	0.682 0.678	0.680	0.601
375	1.320 1.276	1.298	1.219
750	2.161 2.197	2.179	2.100
1500	2.673 2.681	2.677	2.598

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human IGF-2 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human IGF-2.	1 plate
Human IGF-2 Standard	Recombinant human IGF-2 in a buffered base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human IGF-2 Detection Antibody	Biotinylated human IGF-2 antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Streptavidin-HRP B(40×)	40× Streptavidin conjugated to horseradish peroxidase.	1 vial
Calibrator Diluent (2×)	A 2× concentrated buffered diluent used to dilute standard and samples.	1 vial
Detection Antibody Diluent (2×)	A 2× concentrated buffered diluent used to dilute detection antibody.	1 vial
Pretreatment buffer A (1×)	1×buffered base used to pretreat samples.	1 vial
Pretreatment buffer B (1×)	1×buffered base used to neutralize samples.	1 vial
PBS (10×)	A 10× concentrated PBS	1 vial
Reagent Diluent Concentrate 3 (5×)	A 5× concentrated buffered base used to dilute HRP.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1vial
TMB Substrate	TMB ELISA Substrate Solution/ TMB Substrate Solution	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Sealers	Adhesive strips.	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="10">Opened/ Reconstituted Reagents</td><td>Streptavidin-HRP B</td><td rowspan="7">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>Pretreatment buffer A (1×)</td></tr>
<tr><td>Pretreatment buffer B (1×)</td></tr>
<tr><td>PBS (10×)</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>Calibrator Diluent (2×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Detection Antibody Diluent (2×)</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>Reagent Diluent Concentrate 3 (5×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent Diluent 3 (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.
- Test tubes for dilution of standards.
- 100mL and 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.
- Human IGF-2 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 Calibrator 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 × 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 (1×).

B. SAMPLE PREPARATION

Follow the sample pretreatment and dilution procedure outlined below in order to dissociate IGF binding proteins from IGF2.

Cell Culture Supernates	**Serum**
To 50 µL of cell culture supernate, add 50 µL of Pretreatment buffer A (1×).	To 10 µL serum, add 95 µL of Pretreatment buffer A (1×).
Mix well.	Mix well.
Incubate 10 minutes at room temperature.	Incubate 10 minutes at room temperature.
Neutralize the acidified sample by adding 50 µL of Pretreatment buffer B (1×).	Neutralize the acidified sample by adding 95 µL of Pretreatment buffer B (1×).
Mix well.	Mix well.
Prior to assay, cell culture supernate samples recommend a 2-fold dilution. A suggested 2-fold dilution is 100 µL of pretreated sample + 100 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.	Prior to assay, serum samples recommend a 20-fold dilution due to high endogenous levels. A suggested 20-fold dilution is 10 µL of pretreated sample + 190 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.
The concentration read off the standard curve must be multiplied by the dilution factor, 6.	The concentration read off the standard curve must be multiplied by the dilution factor, 400.

* Pretreated samples must be assayed in 60 minutes. Do not freeze pretreated samples.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: Human IGF-2 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 (1×) - Use deionized or distilled water to prepare Calibrator Diluent (1×).

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

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

Reagent Diluent 3 (1×) - Dilute Reagent Diluent Concentrate 3 (5×) with PBS (1×) to prepare Reagent Diluent 3 (1×).

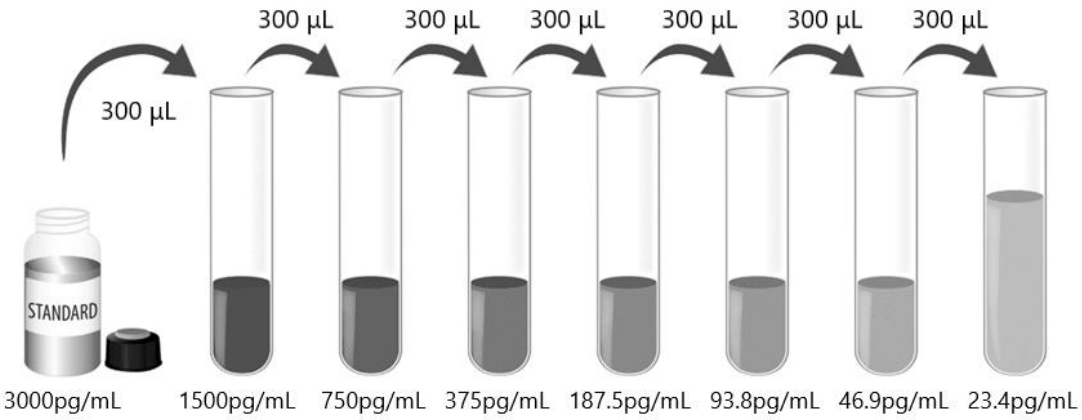
Detection Antibody (1 ×) - Centrifuge briefly before opening. Reconstitution volume refer to vial label to prepare Detection Antibody (100×). Allow the Detection Antibody to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Aliquot and store if needed. Dilute to Detection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

Streptavidin-HRP B (1×) - Centrifuge briefly before opening. Dilute to the working concentration specified on the vial label using Reagent Diluent 3 (1×).

Human IGF-2 Standard - Centrifuge briefly before opening. Refer to the vial label for the reconstitution volume *. This reconstitution produces a stock solution of 3000 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 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 1500 pg/mL standard serves as the high standard. The Calibrator 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

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

Note: Human IGF-2 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**. A plate layout is provided for a record of standards and samples assayed. (Samples may require pretreatment and 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 2 hours at room temperature**.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of the working dilution of Streptavidin-HRP B to each well. Cover the plate and **incubate for 20 minutes at room temperature. 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 30 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 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 IGF-2 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 activated and diluted, the concentration read from the standard curve must be multiplied by the activation and dilution factor. (For example, Activated Cell culture (3 times) requires 2 times dilution with Calibrator Diluent (1×), and the concentration must be multiplied by the activation and dilution factor of 6. Activated Serum samples (20 times) requires 20 times dilution with Calibrator Diluent (1×), and the concentration must be multiplied by the activation and dilution factor of 400.)

VIII. REFERENCES

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产品信息及操作手册

人 IGF-2 Valukine™ ELISA 试剂盒

目录号：VAL160

适用于定量检测天然和重组人 IGF-2 的浓度

科研专用，不可用于临床诊断

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版本号 202410.3

目录

I. 背景

胰岛素生长因子-2 (IGF2)，也称为生长激素-A，是一种8 kDa糖基化的胰岛素家族肽激素。它是生长和代谢调节蛋白系统的一部分，在神经系统、肾上腺皮质和骨骼系统的发育过程中尤为重要 (1,2)。人IGF2作为前体蛋白合成，包含24个氨基酸 (aa) 信号序列、67个aa的成熟区，和89个aa的C-末端前段，通常称为E结构域(3,4)。连续的蛋白水解过程产生18 kDa的前体IGF2，然后是成熟的IGF2，它由一个N端B结构域、一个C结构域、一个A结构域和一个D结构域组成 (5,6)。B-C-A序列是前胰岛素的再融合序列，它被进一步处理去除C结构域。成熟的人IGF2与小鼠和大鼠IGF2的氨基酸同源性分别为91%和94%。其他与IGF2相关的产物包括一个56 aa的N末端延伸的剪接异构体，“大”IGF2保留21 aa的E结构域，以及Preptin (E结构域的34 aa片段)，其由胰腺β细胞分泌，并促进胰岛素分泌和成骨细胞增殖 (7-9)。

IGF2主要由肝脏合成，在胎儿和成人体内循环 (1,2)。在血液中，成熟的IGF2与IGFBP-1、2、4或6结合，并可与IGFBP-3或IGFBP-5加ALS（酸性不稳定亚单位）形成三元复合物。它还可以与可溶性IGF2 R结合 (可能携带20%的总循环IGF2) (10,11)。ProIGF2也可以循环并与IGFBP-5和ALS形成三元复合物 (6,11)。一旦与其载体蛋白分离，成熟的IGF2在多种细胞类型具有促有丝分裂、抗凋亡和胰岛素样活性。它结合并激活IGF-1R、IGF2R、胰岛素R (A和B异构体) 和IGF-1R：胰岛素R-A混合受体 (6,10,12-15)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人IGF-2捕获抗体包被于微孔板上，样品和标准品中的人IGF-2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IGF-2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）	38.8	176.9	676.0	39.8	178.5	682.8
标准差	2.5	14.0	45.8	2.7	13.1	42.7
CV%	6.4	7.9	6.8	6.7	7.3	6.3

B. 回收率

在细胞培养基中掺入检测范围内不同水平的人 IGF-2，测定其回收率。回收率范围在 92.0-104.7%，平均回收率在 97.4%。

C. 灵敏度

人 IGF-2 的最低可测剂量（MDD）一般小于 1.24 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 生产的大肠杆菌表达的高纯度重组人 IGF-2 蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人 IGF-2，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	平均期待值（%）	范围（%）
1:2	100.1	93.3-111.8
1:4	105.2	93.4-121.4
1:8	109.7	93.3-124.8
1:16	100.0	91.5-115.6

F. 样本值

人血清样本 - 使用本试剂盒检测了 7 份人血清样本中人 IGF-2 的水平。所有样本的检测值范围为 177.3-452.6 ng/mL，平均值为 343.1 ng/mL。

G. 特异性

将以下因子配制成 50 ng/mL 的浓度检测，没有观察到明显的交叉反应或干扰。

Recombinant human	
IGFBP-rp1	IGF2R
IGF-1	Insulin R
IGF1R	Proinsulin

含有 3.1 ng/mL 重组小鼠 IGF-2 的样本检测值为 42.3 pg/mL（1.4%交叉反应性）。

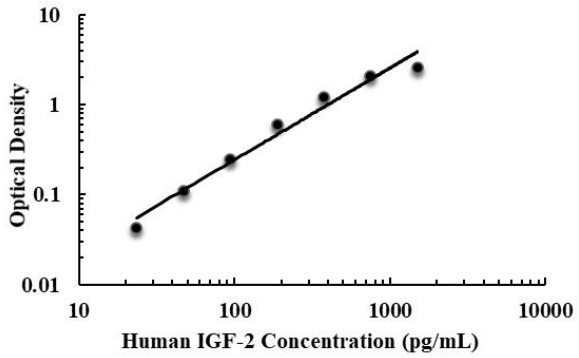
以下因子不会发生交叉反应，但在下列浓度下会产生干扰：

Recombinant Factor	**Concentration**
human IGFBP-1	> 2500 pg/mL
human IGFBP-2	> 12.5 pg/mL
human IGFBP-3	> 0.8 pg/mL
human IGFBP-4	> 125 pg/mL
human IGFBP-5	> 1563 pg/mL
human IGFBP-6	> 0.8 pg/mL

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	0.080 0.078	0.079	-
23.4	0.123 0.121	0.122	0.043
46.9	0.187 0.188	0.188	0.109
93.8	0.329 0.327	0.328	0.249
187.5	0.682 0.678	0.680	0.601
375	1.320 1.276	1.298	1.219
750	2.161 2.197	2.179	2.100
1500	2.673 2.681	2.677	2.598

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human IGF-2 Microplate	包被抗人 IGF-2 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human IGF-2 Standard	人 IGF-2 标准品（冻干粉），参考瓶标签进行重溶	2 瓶
Human IGF-2 Detection Antibody	生物素化的抗人 IGF-2 检测抗体，冻干粉，参考瓶标签进行重溶	1 瓶
Streptavidin-HRP B (40×)	40×浓缩的链霉亲和素标记的 HRP	1 瓶
Calibrator Diluent (2×)	浓缩的标准品稀释液（2×）用于稀释标准品和样本	1 瓶
Detection Antibody Diluent (2×)	浓缩的检测抗体稀释液（2×）用于稀释检测抗体	1 瓶
Pretreatment buffer A (1×)	样本预处理液 A（1×）	1 瓶
Pretreatment buffer B (1×)	样本预处理液 B（1×）	1 瓶
PBS (10×)	浓缩 PBS（10×）	1 瓶
Reagent Diluent Concentrate 3 (5×)	浓缩的试剂稀释液 3（5×）用于稀释 HRP	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="10">已打开，稀释或重溶的试剂</td><td>链霉亲和素-HRP B</td><td rowspan="8">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>样本预处理液 A（1×）</td></tr>
<tr><td>样本预处理液 B（1×）</td></tr>
<tr><td>PBS (10×)</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>标准品稀释液（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，多余的丢弃</td></tr>
<tr><td>检测抗体稀释液（2×）</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，多余的丢弃</td></tr>
<tr><td>试剂稀释液 3（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 3，多余的丢弃</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

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
- 用来稀释标准品的试管
- 100 mL 及 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，使用时请穿戴防护手套、衣服、眼镜和脸的保护罩。使用后请彻底洗手。
- 唾液中含有人 IGF-2，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。样本可能需要用标准品稀释液（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 × 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（1×）稀释。

B. 样本准备工作

按照下面列出的样品预处理和稀释程序，将IGF结合蛋白从IGF2中分离出来。

细胞培养上清	**血清**
50 µL 细胞培养上清中加入50 µL 的样本预处理液 A（1×）	10 µL 的血清中加入95 µL的样本预处理液 A（1×）
混匀	混匀
室温孵育10 min	室温孵育10 min
加入50 µL的样本预处理液 B（1×）中和样本	加入95 µL的样本预处理液 B（1×）中和样本
混匀	混匀
检测样本前建议用标准品稀释液（1×）2倍稀释后进行检测，例如：100 µL预处理后的细胞上清样本 + 100 µL标准品稀释液。最佳稀释度应由最终用户确定。	检测样本前建议用标准品稀释液（1×）20倍稀释后进行检测，例如：10 µL预处理后的血清样本 + 190 µL标准品稀释液。最佳稀释度应由最终用户确定。
读取的浓度乘以稀释倍数6	读取的浓度乘以稀释倍数400

***处理后的样本必须在60分钟内进行检测，不可冷冻已处理的样本。**

C. 检测前准备工作

使用前请将所有试剂放置于室温。
注：*唾液中含有人 IGF-2，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×）用蒸馏水或去离子水稀释配制成 500 mL 工作浓度的洗涤液（1×）。

标准品稀释液（1×）：使用蒸馏水或去离子水稀释配制成标准品稀释液（1×）。

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

PBS（1×）：使用蒸馏水或去离子水稀释配制成 PBS（1×）。

试剂稀释液 3（1×）：使用 PBS（1×）稀释配制成试剂稀释液 3（1×）。

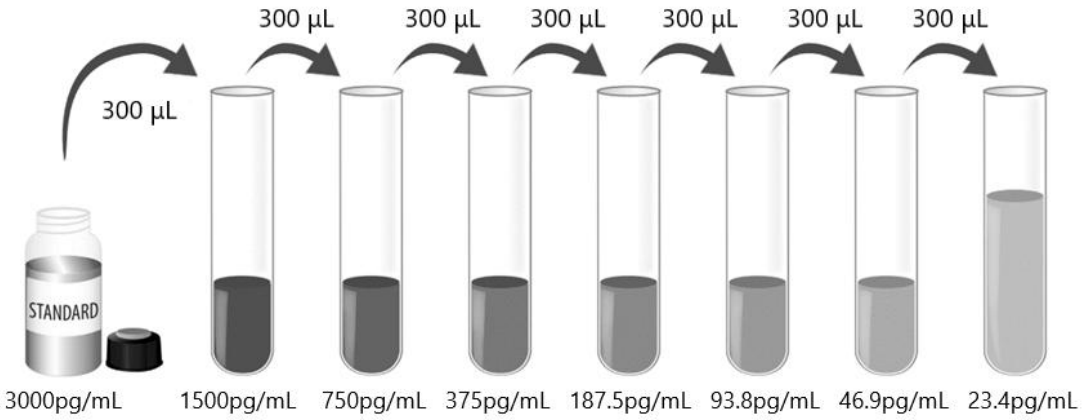
检测抗体（1×）：开盖前请瞬时离心。参考检测抗体瓶标签重溶冻干粉，制备检测抗体（100×）。轻轻震摇至少 15 分钟，其充分溶解。如有需要分装保存。用检测抗体稀释液（1×）稀释至检测抗体（1×），至少在使用前 15 分钟准备。

链霉亲和素-HRP B（1×）：开盖前请瞬时离心。用试剂稀释液 3（1×）将链霉亲和素-HRP B（40×）稀释至工作浓度。

人 IGF-2 标准品：开盖前请瞬时离心。参照冻干标准品瓶身注明的方式重溶冻干标准品*，得到浓度为 3000 pg/mL 标准品母液。轻微震摇至少 15 分钟，使其充分溶解。

***如有疑问，请咨询我们的技术支持。**

每个稀释管中加入 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。1500 pg/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 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。

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。

注：唾液中含有人 IGF-2，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；（样本需要预处理和稀释，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 B 工作液。用封板膜封住反应孔，**室温孵育 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，**室温孵育 30 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 IGF-2 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。

    如果样品被活化和稀释，从标准曲线读取的浓度必须乘以活化和稀释的倍数。（如：细胞上清样本活化（3 倍）后需要用标准品稀释液（1×）2 倍稀释后进行检测，则浓度必须乘以活化和稀释系数 6；血清样本活化（20 倍）后需要用标准品稀释液（1×）20 倍稀释后进行检测，则浓度必须乘以活化和稀释系数 400。）

VIII. 参考文献

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.