PRODUCT INFORMATION & MANUAL

Glucagon Valukine™ ELISA

VAL150

For the quantitative determination of natural and recombinant Glucagon 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

Version202201.1

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I. BACKGROUND

Glucagon is a 29 amino acid (aa) peptide produced by the pancreas that plays a critical role in glucose metabolism and homeostasis (1-4). The Glucagon precursor mRNA is expressed by alpha cells (α-cells) of the pancreas, L cells of the intestine, and in the brain (1, 2). Only the pancreatic α-cells express the prohormone convertase PC2, also called PCSK2, which is required to produce Glucagon (2). Intestinal L cells instead express the prohormone convertase PC1, which processes the precursor to the Glucagon-overlapping peptides glicentin and oxyntomodulin. L cells also produce two Glucagon-like peptides, GLP-1 and GLP-2 that are derived from the same Glucagon precursor and influence glucose metabolism, but do not share any common sequence with Glucagon (1, 2). The aa sequence of the mature Glucagon peptide is identical in human, mouse, rat, pig, dog, horse, cow, sheep, and Xenopus.

In normal metabolism, Glucagon is secreted in response to low blood glucose (hypoglycemia) and downregulated in response to high blood glucose (hyperglycemia). Although Glucagon binding sites are found in liver, brain, pancreas, kidney, intestine, and adipose tissue, the main activity of Glucagon receptors occurs in the liver, where Glucagon stimulates gluconeogenesis and glycogenolysis, thereby increasing blood glucose (1-4). It is particularly important that the brain receive sufficient glucose, since it is unable to store more than a minute quantity. Therefore the release of Glucagon from α-cells is under control by both hormones and neurotransmitters, and is very responsive to circulating glucose concentration. Insulin, and/or the zinc that islet β cells secrete with it, downregulates Glucagon secretion in intact islets (5, 6). Glucagon secretion is also downregulated by the neurotransmitter γ-aminobutyric acid (GABA), somatostatin produced by islet δ-cells, and GLP-1, but is enhanced by the neurotransmitter L-glutamate, amino acids (especially arginine), and Glucagon itself (2-4, 7). Through receptors on the α-cells, these substances affect potassium, sodium, and calcium channel activity and alter intracellular calcium concentration (2-4). Glucose suppression of Glucagon secretion is probably indirect, acting through paracrine signals from other islet cells (8).

Like insulin, Glucagon is dysregulated in type 2 diabetes (T2D) and contributes to its pathology (2-4). Glucagon secretion is less responsive to insulin-mediated

suppression in times of high circulating glucose, causing glucagonemia, and increasing the risk of hyperglycemia. Glucagon is also regulated by some of the same messengers that regulate insulin (10-12). Leptin inhibits α-cell glucagon secretion and stimulates β-cell insulin secretion, but glucagon blunts the leptin-mediated insulin secretion (10). Islet α-cells express ghrelin receptors and respond to ghrelin by increasing Glucagon secretion (11). Glucocorticoids, activated by 11β-HSD1, depress Glucagon secretion in hypoglycemia and insulin secretion in hyperglycemia (12). Although genetic polymorphisms of the Glucagon receptor are associated with T2D, downregulation of Glucagon secretion or deletion of the Glucagon receptor in mice that are susceptible to T2D actually improves glycemic control (13, 14).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Glucagon has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Glucagon present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for Glucagon are pipetted into the wells. After washing away any unbound substances, streptavidin-HRP are pipetted into the wells. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Glucagon 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 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)	1000.1	2929.6	8594.7	1000.9	2933.3	8639.8
Standard Deviation	20.9	44.8	172.8	19.6	39.5	173.8
CV%	2.1%	1.5%	2.0%	2.0%	1.3%	2.0%

B. RECOVERY

The recovery of Glucagon spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 111.4-120% with an average of 114.8%.

The recovery of Glucagon spiked to different levels throughout the range of the assay in human serum was evaluated. The recovery ranged from 80.8-113.6% with an average of 99.4%.

C. SENSITIVITY

The minimum detectable dose (MDD) of Glucagon is typically less than 10.26 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 Glucagon (aa53-81) highly-purified from porcine pancreas.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of Glucagon 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	101.1	91.7-108.7
1:4	101.6	91.0-112.5
1:8	105.7	94.1-115.2
1:16	85.7	72.6-101.9

F. SAMPLE VALUES

Serum - Seven human serum samples were evaluated for the presence of Glucagon in this assay. All samples measured less than the lowest Glucagon standard, 500 pg/mL.

G. SPECIFICITY

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

Glucentin-Related Polypeptide (aa 21-50)	Glucagon-Like Peptide 1 (aa 1-37)
Glucagon-Like Peptide 2 (aa 146-178)	Glucagon-Like Peptide 1 (aa 7-37)
	Glucagon-Like Peptide 1 (aa 92-128)

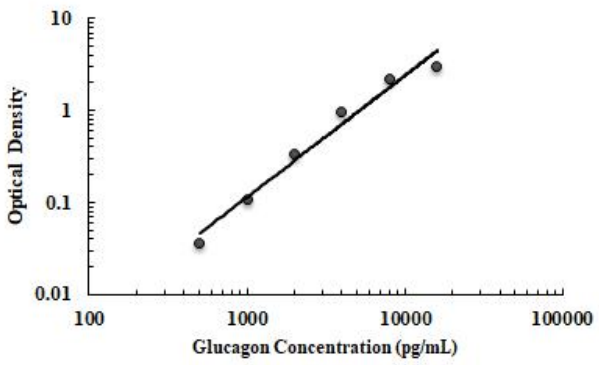
Glucagon (aa 71-81) does not cross-react in this assay but does interfere at concentrations > 25 ng/mL.

A sample containing 1.6 ng/mL of Oxyntomodulin (aa 53-89) reads as 469 pg/mL (30% cross-reactivity).

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	0.027 0.028	0.028	-
500	0.063 0.065	0.064	0.037
1000	0.133 0.139	0.136	0.109
2000	0.358 0.365	0.362	0.334
4000	0.982 1.005	0.994	0.966
8000	2.239 2.277	2.258	2.231
16000	2.983 2.986	2.985	2.957

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Glucagon Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a Mouse antibody against Glucagon.	1 plate
Glucagon Standard	Glucagon in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Glucagon Detection Antibody	Biotinylated Glucagon antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Assay Diluent (1×)	A buffered protein base with preservatives.	1 vial
Calibrator Diluent (1×)	Diluent used to dilute standard and samples.	2 vials
Detection Antibody Diluent (4×)	Concentrated buffered diluent used to dilute Detection Antibody.	1 vial
Streptavidin-HRP B (40×)	40× Streptavidin conjugated to horseradish peroxidase.	1 vial
Reagent Diluent (10×)	A 10× concentrated buffered protein base used to dilute Detection Antibody and HRP.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
Color Reagent A	Stabilized hydrogen peroxide.	1 vial
Color Reagent B	Stabilized chromogen (tetramethylbenzidine).	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="10">Opened/ Reconstituted Reagents</td><td>Streptavidin-HRP B</td><td rowspan="5">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Assay Diluent (1×)</td><td>Prepare fresh for each assay.</td></tr>
<tr><td>Standard</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Detection Antibody</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>Reagent Diluent (10×)</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent (1×)</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>Detection Antibody Diluent (4×)</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>
<tr><td>Microplate Wells</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.
- Horizontal orbital microplate shaker capable of maintaining a speed of 500±50 rpm.
- 500 mL graduated cylinder.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.
- 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 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 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

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

Serum samples require a 4-fold dilution prior to the assay. 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 - 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.

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

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

Detection Antibody- Reconstitution Volume refer to vial label with Reagent Diluent (1×). Aliquot and store if needed. Dilute stock solution in Detection Antibody Diluent (1×) to the working concentration of 50 ng/mL. Prepare at least 15 minutes prior to use.

Streptavidin-HRP B (1×) **-** Dilute to the working concentration specified on the vial

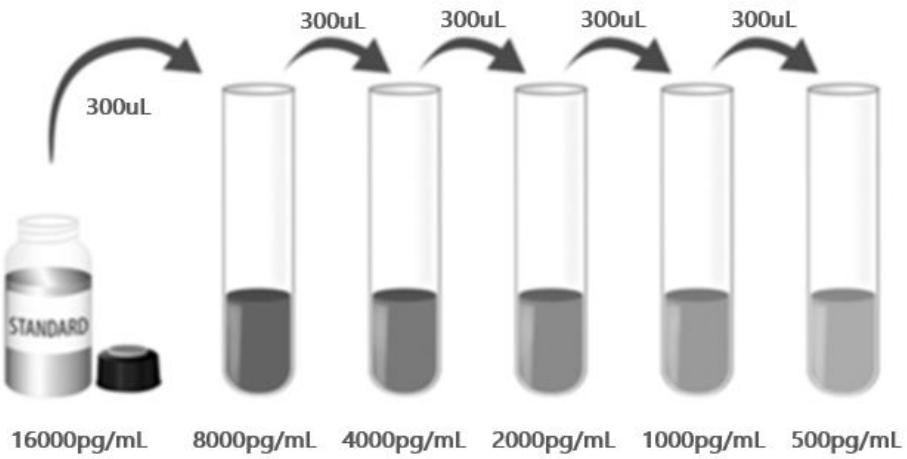
label using Reagent Diluent (1×).

Substrate Solution - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Glucagon Standard - Refer to the vial label for the reconstitution volume*. This reconstitution produces a stock solution of 16000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

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

Pipette 300 µL of 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 16000 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.
- Substrate Solution should remain colorless until added to the plate. Keep

Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.

- Stop Solution should be added to the plate in the same order as the Substrate Solution. 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 Substrate Solution.

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 50 µL of Assay Diluent to each well.
4. Add 50 µL of standard, or prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500±50rpm.** A plate layout is provided for a record of standards and samples assayed. (Samples may require dilution. See Sample Preparation section.)
5. 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.
6. Add 100 µL of the Detection Antibody diluted in Detection Antibody Diluent, to each well. **Cover with a new adhesive strip and incubate for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500±50rpm.**
7. Repeat the aspiration/wash as in step 5.
8. 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 on a horizontal orbital microplate shaker set at 500 ± 50rpm. Avoid placing the plate in direct light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. **Incubate for 20 minutes at room temperature on a horizontal orbital microplate shaker set at 500 ± 50rpm. Avoid placing the plate in direct light.**
11. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough

mixing.

12. Determine the optical density of each well immediately, 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.

13. **CALCULATION OF RESULTS**

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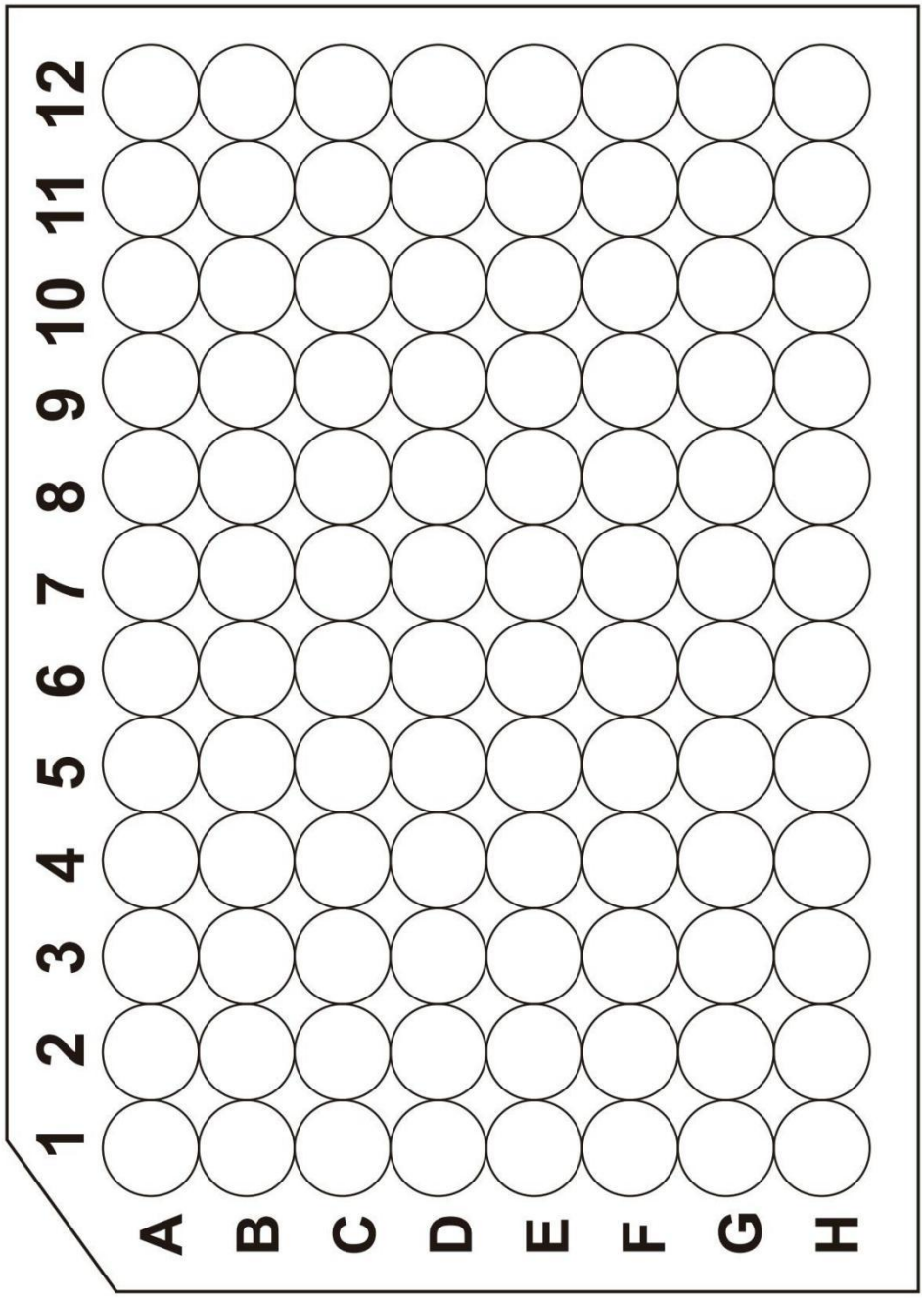
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

Glucagon Valukine™ ELISA 试剂盒

目录号：VAL150

适用于定量检测天然和重组 Glucagon 的浓度

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目录

I. 背景

胰高血糖素是由胰腺产生的一种29氨基酸(aa)肽，在葡萄糖代谢和体内平衡中起着关键作用(1-4)。胰高血糖素前体mRNA由胰腺的α细胞(α细胞)、肠道的L细胞和大脑表达(1, 2)。只有胰腺α细胞表达激素原转化酶PC2，也称为PCSK2，这是产生胰高血糖素所必需的(2)。肠道L细胞反而表达激素转化酶原PC1，该酶处理胰高血糖素重叠肽，生长激素释放肽和氧调节蛋白的前体。L细胞也产生两种胰高血糖素样肽，GLP-1和GLP-2，它们来源于同一胰高血糖素前体并影响葡萄糖代谢，但与胰高血糖素没有任何共同序列(1, 2)。成熟胰高血糖素肽的aa序列在人、小鼠、大鼠、猪、狗、马、牛、羊和爪蟾中是相同的。

在正常代谢中，胰高血糖素在低血糖时分泌，在高血糖时下调。尽管胰高血糖素结合位点存在于肝、脑、胰腺、肾、肠和脂肪组织中，但胰高血糖素受体的主要活性发生在肝脏，在肝脏中，胰高血糖素刺激糖异生和糖原分解，从而增加血糖大脑获得足够的葡萄糖尤其重要，因为它不能储存超过一分钟的葡萄糖(1-4)。因此，α细胞释放胰高血糖素受到激素和神经递质的控制，并且对循环葡萄糖浓度非常敏感。胰岛素或胰岛β细胞分泌的锌可下调完整胰岛的胰高血糖素分泌(5, 6)。胰高血糖素的分泌也被神经递质γ-氨基丁酸（GABA）、胰岛δ细胞产生的生长抑素和GLP-1下调，但被神经递质L-谷氨酸、氨基酸和胰高血糖素本身增强(2-4, 7)。通过α细胞上的受体，这些物质影响钾、钠和钙通道活性，并改变细胞内钙浓度(2-4)。葡萄糖对胰高血糖素分泌的抑制可能是间接的，通过来自其他胰岛细胞的旁分泌信号起作用(8)。

与胰岛素一样，胰高血糖素在2型糖尿病(T2D)中失调，并参与其病理过程(2-4)。在高循环血糖时，胰高血糖素分泌对胰岛素介导的抑制反应较弱，导致胰高血糖素血症，并增加高血糖风险。胰高血糖素也由一些调节胰岛素的信使调节(10-12)。瘦素抑制α细胞胰高血糖素分泌并刺激β细胞胰岛素分泌，但胰高血糖素使瘦素介导的胰岛素分泌变迟钝(10)。胰岛α细胞表达生长激素释放肽受体，并通过增加胰高血糖素分泌对生长激素释放肽作出反应(11)。糖皮质激素由11β-HSD1激活，在低血糖症中抑制胰高血糖素分泌，在高血糖症中抑制胰岛素分泌(12)。尽管胰高血糖素受体的遗传多态性与T2D相关，但对T2D敏感的小鼠胰高血糖素分泌的下调或胰高血糖素受体的缺失实际上改善了血糖控制(13, 14)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。小鼠抗Glucagon抗体包被于微孔板上，样品和标准品中的Glucagon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小鼠抗Glucagon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 (pg/mL)	1000.1	2929.6	8594.7	1000.9	2933.3	8639.8
标准差	20.9	44.8	172.8	19.6	39.5	173.8
CV%	2.1%	1.5%	2.0%	2.0%	1.3%	2.0%

B. 回收率

在细胞培养基样本中掺入检测范围内不同水平的人Glucagon，测定其回收率。回收率范围在111.4-120%，平均回收率在114.8%。

在人血清样本中掺入检测范围内不同水平的人Glucagon，测定其回收率。回收率范围在80.8-113.6%，平均回收率在99.4%。

C. 灵敏度

Glucagon的最低可测剂量（MDD）一般小于10.26pg/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生产的来自猪胰腺的高纯度Glucagon (aa53-81)蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的Glucagon，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	101.1	91.7-108.7
1:4	101.6	91.0-112.5
1:8	105.7	94.1-115.2
1:16	85.7	72.6-101.9

F. 样本预值

血清样本 - 使用本试剂盒检测了7份人血清样本中人Glucagon的水平。7份样本的检测值均低于最低的Glucagon标准品500pg/mL。

G. 特异性

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

Glucentin-Related Polypeptide (aa 21-50)	Glucagon-Like Peptide 1 (aa 1-37)
Glucagon-Like Peptide 2 (aa 146-178)	Glucagon-Like Peptide 1 (aa 7-37)
	Glucagon-Like Peptide 1 (aa 92-128)

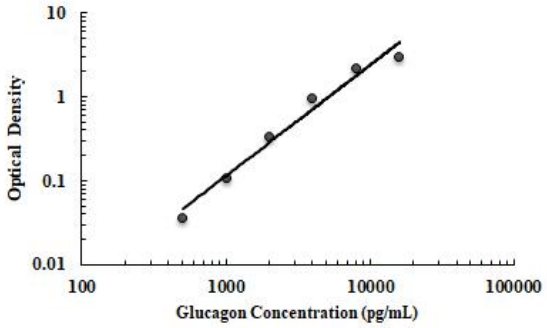
Glucagon(aa 71-81)在本试验中不发生交叉反应，但在浓度>25 ng/mL时发生交叉反应。

含有1.6ng/mL Oxyntomodulin（aa 53-89）的样本读数为469 pg/mL（30%交叉反应性）。

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	0.027 0.028	0.028	-
500	0.063 0.065	0.064	0.037
1000	0.133 0.139	0.136	0.109
2000	0.358 0.365	0.362	0.334
4000	0.982 1.005	0.994	0.966
8000	2.239 2.277	2.258	2.231
16000	2.983 2.986	2.985	2.957

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Glucagon Microplate	包被小鼠抗 Glucagon 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Glucagon Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Glucagon Detection Antibody	生物素化的 Glucagon 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Assay Diluent (1×)	检测缓冲液（1×）	1 瓶
Calibrator Diluent (1×)	标准品稀释液（1×）	2 瓶
Detection Antibody Diluent (4×)	浓缩的检测抗体稀释液（4×）	1 瓶
Streptavidin-HRP B (40×)	40×浓缩的链霉亲和素标记的 HRP	1 瓶
Reagent Diluent (10×)	浓缩的试剂稀释液（10×）	1 瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1 瓶
Color Reagent A	显色液 A	1 瓶
Color Reagent B	显色液 B	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="6">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>显色液 A</td></tr>
<tr><td>显色液 B</td></tr>
<tr><td>检测缓冲液（1×）</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*</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（1×）</td><td>2-8℃储存，最多 30 天*</td></tr>
<tr><td>检测抗体稀释液（4×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×检测抗体稀释液</td></tr>
<tr><td>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×试剂稀释液</td></tr>
<tr><td>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450nm检测波长的吸收值及540nm或570nm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
- 振荡器（速度可调至500±50rpm）
- 500mL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。
- Color Reagent B可能引起皮肤、眼睛和呼吸道刺激，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。

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 x g离心15分钟。吸取血清样本之后即刻用于检测，或者分装，-20℃贮存备用。避免反复冻融。样本可能需要用标准品稀释液（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：

50μL细胞上清液+150μL标准品稀释液（1×）。

血清样本需要用标准品稀释液（1×）4倍稀释后进行检测，例如：

50μL血清样本+150μL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：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20mL浓缩洗涤液用去离子水或蒸馏水稀释配制成500mL工作浓度的洗涤液。

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

试剂稀释液（1×）：使用去离子水或蒸馏水稀释配制成试剂稀释液（1×）。

检测抗体：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。再用检测抗体稀释液（1×）稀释至工作浓度50ng/mL，至少在使用前15分钟准备。

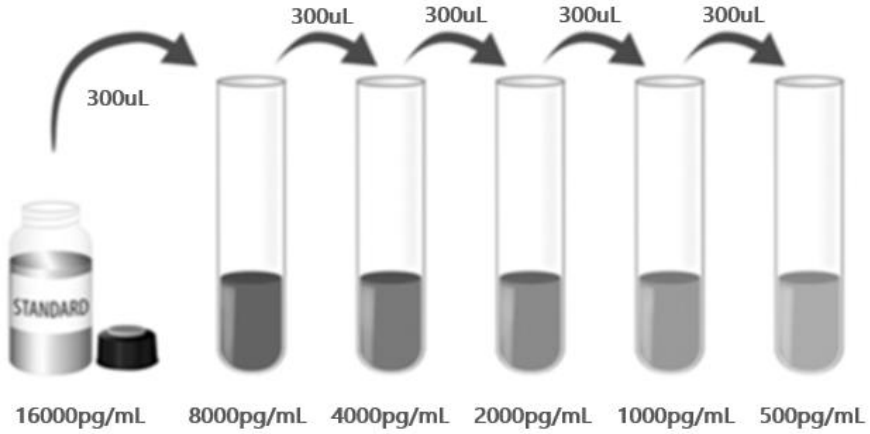
链霉亲和素- HRP B：用试剂稀释液（1×）将链霉亲和素- HRP B（40×）稀释至工作浓度链霉亲和素- HRP B（1×）。

显色剂：按试验所需用量（100μL/孔）将显色液A和显色液B等体积混合，避光保存，现用现配，须在15分钟内使用。

Glucagon标准品：冻干标准品的重溶体积请参考瓶身标签，得到浓度为16000pg/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300μL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。16000pg/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（0pg/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；
- 混合后的显色底物在上板前应为无色，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显色底物上板顺序一致；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. 在每个微孔内加入50μL检测缓冲液；
4. 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50μL。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。）
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400μL，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100μL配制好的检测抗体。用封板胶纸封住反应孔，**室温500±50rpm水平振荡孵育2小时**；
7. 重复第5步洗板操作；
8. 在每个微孔内加入100μL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500±50rpm水平振荡孵育20分钟。注意避光；**
9. 重复第5步洗板操作；
10. 在每个微孔内加入100μL显色剂，**室温500±50rpm水平振荡孵育20分钟。注意避光**；
11. 在每个微孔内加入50μL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；
12. 加入终止液后30分钟内，使用酶标仪测量450nm的吸光度值，设定540nm或570nm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；
13. **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Glucagon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