PRODUCT INFORMATION & MANUAL

Human Insulin Valukine™ ELISA

Catalog Number: VAL146

For the quantitative determination of natural and recombinant Human Insulin 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 202407.4

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I. BACKGROUND

Insulin is a peptide hormone of the insulin-like peptide family that also includes insulin-like growth factors (IGFs), relaxins, and other insulin-like peptides (1-3). Its production by pancreatic β cells is essential for glucose metabolism and regulation of energy balance. Failure of insulin control causes diabetes mellitus (DM), which can either be of Type I (T1D, 5% of diagnosed DM), previously called juvenile or insulin-dependent diabetes, or Type II (T2D, 95% of diagnosed DM), previously called adult-onset or insulin-independent diabetes (4). T1D is a primary insufficiency of β cell insulin production while T2D is a functional insulin deficiency caused mainly by insulin resistance of the target cells. Mutations of the insulin gene are a cause of neonatal diabetes (2, 5, 6). DM has become more frequent over time, and in 2011 affected 79 million adults in the US (8% of the population). Frequency increases with age (27% of adults >65 years) and obesity, and can vary with ethnic background (4). DM is the seventh leading cause of death in the US (2, 4). Complications include heart disease, stroke, hypertension, blindness, kidney disease, and neuropathy (4).

Insulin is synthesized as a 110 amino acid (aa) preproprotein. A 24 aa signal sequence is cleaved to form the 86 aa proinsulin peptide, which undergoes further proteolysis to generate the 30 aa B chain (aa 25–54 of the preproprotein), the 21 aa A chain (aa 90-110), and the 34 aa intervening C-peptide (1, 2). Mature human insulin is the disulfide-linked heterodimer of A and B chains, which shares 98% aa sequence identity (50/51 aa) with porcine and canine insulin, 94% (48/51 aa) with bovine insulin, and 92% (47/51 aa) with mouse, rat, feline and ovine insulin. Insulin is stored in β cells as a zinc-coordinated hexamer (2). It is released as a zinc-free monomer into the hepatic portal vein, thus achieving its highest concentration in the liver (2, 7). Basal levels are continuously secreted, with higher secretion stimulated by food ingestion (7). In diabetics requiring insulin replacement or supplementation (26% of those diagnosed with diabetes), natural insulin or a synthetic analogue is delivered subcutaneously via injection or an implanted pump (4, 7).

Insulin acts through the insulin receptor (Ins R), a receptor tyrosine kinase that is present in varying quantities on all cells. Insulin initiates downstream signaling by binding and cross-linking two receptor molecules to form a trimeric receptor/ligand complex (2, 3, 8). Signals facilitate the cellular uptake of glucose by regulating the appearance of membrane glucose transporters. The Ins R is present in two isoforms, A and B, which may homodimerize or heterodimerize with the IGF-I receptor (3, 6). All receptor combinations bind insulin, IGF-I or IGF-II, but with differing affinities (3, 6). This system allows fine tuning of insulin-mediated signaling pathway activation according to the concentrations of insulin, IGF-I and IGF-II, and expression of receptor subunits on the cell surface (6).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Insulin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human Insulin present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for human Insulin 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 Insulin 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.
- 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-C (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 (pmol/L)	383.6	178.4	69.6	386.8	178.4	61.6
Standard Deviation	20.4	4.4	6.4	19.6	4.0	4.4
CV%	5.3	2.5	9.3	5.1	2.1	7.0

B. RECOVERY

The recovery of human Insulin spiked to different levels throughout the range of the assay in cell culture supernate was evaluated. The recovery ranged from 88.2 to 117.1% with an average of 106.1%.

C. SENSITIVITY

The minimum detectable dose (MDD) of human Insulin is typically less than 0.14 pmol/L.

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 *S.cerevisiae*-expressed recombinant human Insulin 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 Insulin and diluted with **Calibrator Diluent-C (1×)** to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	99.3	86.6-118.5
1:4	104.6	92.3-123.8
1:8	101.5	89.3-113.7
1:16	104.4	95.4-113.4

F. SPECIFICITY

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

Recombinant human	
C-Peptide	Relaxin-1
IGF-I	Relaxin-2
	Relaxin-3

This Valukine™ also recognizes natural canine and porcine Insulin.

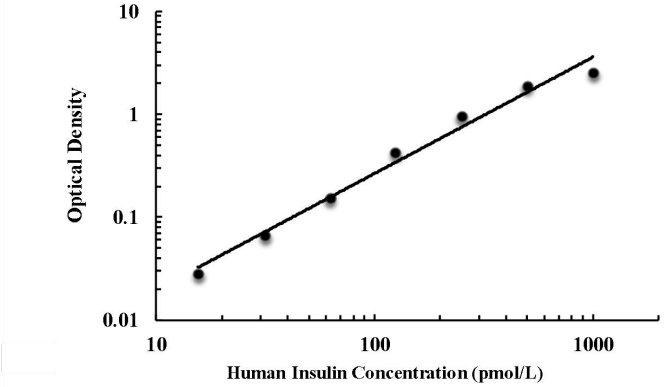
Recombinant human (rh) Insulin R and rhIGF II does not cross react in this assay but does interfere at concentrations > 1.56 and 3.13 ng/mL respectively.

A sample containing 25.0 ng/mL of recombinant human Proinsulin reads as 922 pg/mL (3.7% 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.



pmol/L	OD	Average	Corrected
0	0.009 0.009	0.009	-
15.6	0.035 0.040	0.037	0.028
31.3	0.077 0.074	0.076	0.067
62.5	0.151 0.170	0.161	0.152
125	0.435 0.441	0.438	0.429
250	0.955 0.979	0.967	0.958
500	1.951 1.802	1.877	1.868
1000	2.503 2.543	2.523	2.514

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Insulin Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human Insulin.	1 plate
Human Insulin Standard	Recombinant human Insulin in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human Insulin Detection Antibody	Biotinylated human Insulin antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent-C (1×)	Buffered diluent used to dilute standard and samples.	2 vials
Detection Antibody Diluent-C Concentrate (4×)	A 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 Concentrate (10×)	A 10× concentrated buffered protein base used to dilute HRP.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB ELISA Substrate Solution/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="8">Opened/ Reconstituted Reagents</td><td>Streptavidin-HRP B</td><td rowspan="4">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>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 Concentrate (10×)</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>Calibrator Diluent-C (1×)</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Detection Antibody Diluent-C Concentrate (4×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Detection Antibody Diluent-C (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 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-C (1×).

B. SAMPLE PREPARATION

Cell culture supernate samples recommend a 4-fold dilution. A suggested 4-fold dilution is 50 µL of sample + 150 µL of **Calibrator Diluent-C (1×)**. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *Insulin is 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×).

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

Reagent Diluent (1×) - Use deionized or distilled water to prepare Reagent Diluent (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-C (1×).Prepare at least 15 minutes prior to use.

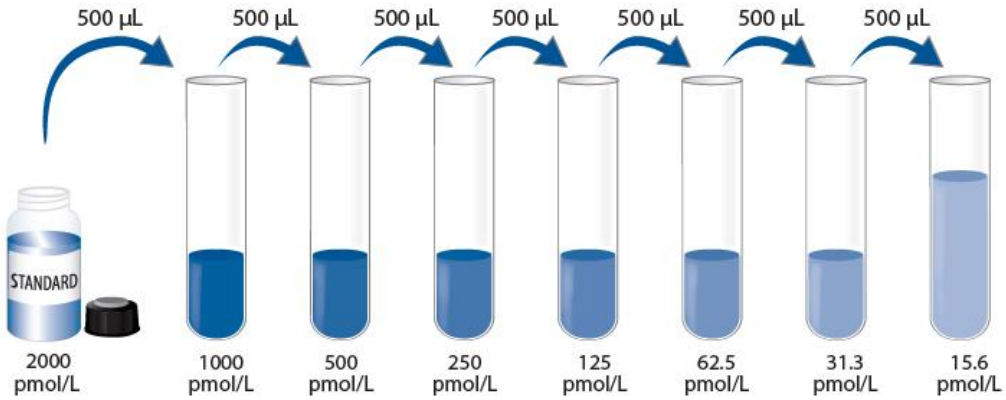
Streptavidin-HRP B (1×) - Centrifuge briefly before opening. Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

Human Insulin Standard - Centrifuge briefly before opening. Refer to the vial

label for the reconstitution volume*.This reconstitution produces a stock solution of 2000 pmol/L. 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 500 µL of **Calibrator Diluent-C (1×)** into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The1000 pmol/L standard serves as the high standard. The **Calibrator Diluent-C (1×)** serves as the zero standard (0 pmol/L).



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: *Insulin is 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 1 hour at room temperature on a horizontal orbital microplate shaker set at 500±50 rpm**. 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 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-C (1×), to each well. Cover with a new adhesive strip and **incubate 1 hour at room temperature on a horizontal orbital microplate shaker set at 500±50 rpm**.
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 on a horizontal orbital microplate shaker set at 500±50 rpm. 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 on a horizontal orbital microplate shaker set at 500±50 rpm**. **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, 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 human Insulin 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.

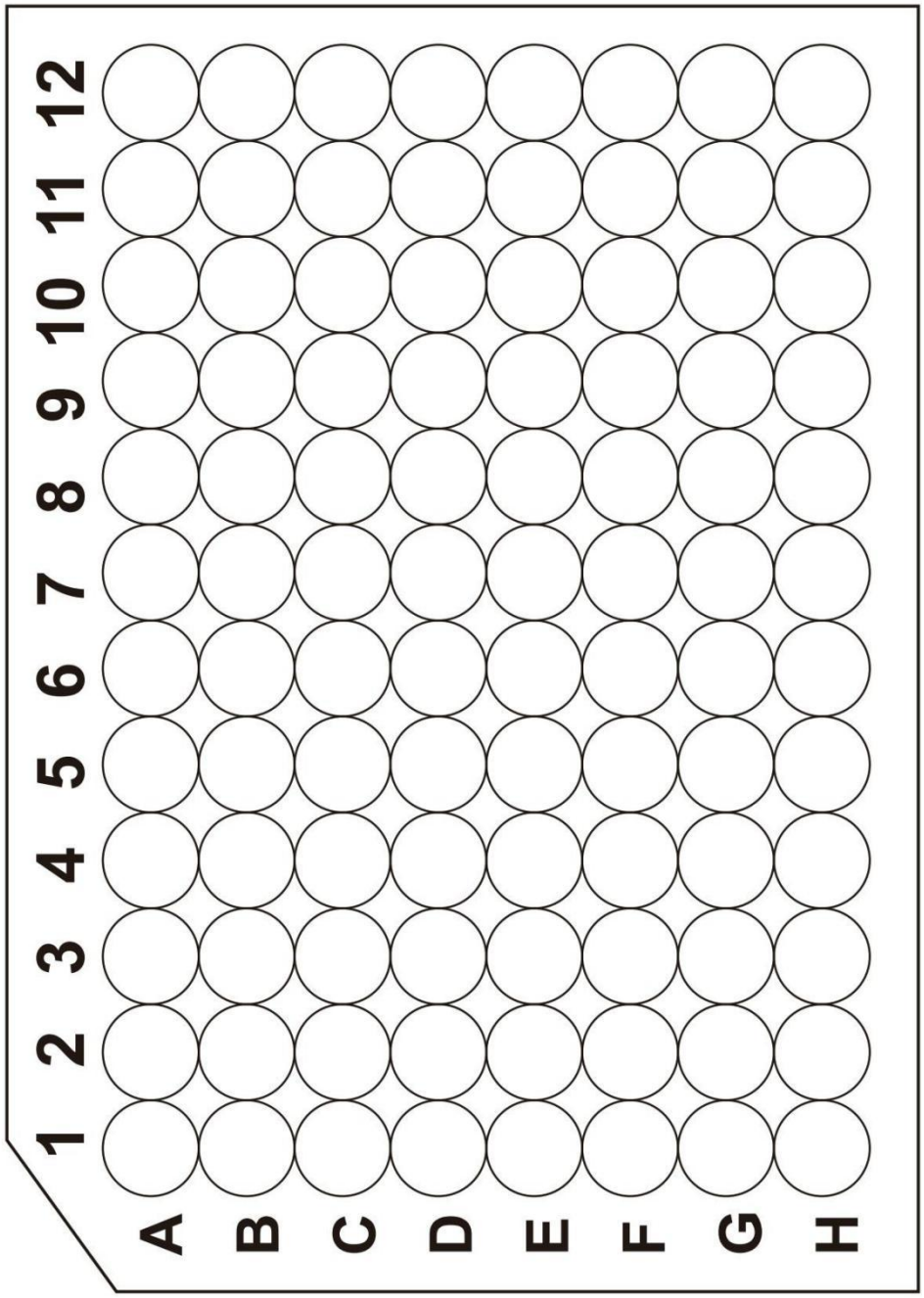
    If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Bell, G.I. et al. (1980) Nature 284:26.
2. Hua, Q. (2010) Protein Cell 1:537.
3. Ward, C.W. and M.C. Lawrence (2009) BioEssays 31:422.
4. https://www.diabetes.org/
5. Stoy, J. et al. (2007) Proc. Natl. Acad. Sci. USA 104:15040.
6. Edghell, E.L. et al. (2008) Diabetes 57:1034.
7. Sheldon, B. et al. (2009) Diabetes Obes. Metab. 11:5.
8. Belfiore, A. et al. (2009) Endocrine Rev. 30:586.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 Insulin Valukine™ ELISA 试剂盒

目录号：VAL146

适用于定量检测天然和重组人 Insulin 的浓度

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

版本号 202407.4

目录

I. 背景

胰岛素（Insulin）是胰岛素样肽家族的肽类激素，也包括胰岛素样生长因子(IGFs)、松弛素和其他胰岛素样肽 (1-3)。它由胰腺β细胞产生，对葡萄糖代谢和能量平衡的调节至关重要。胰岛素控制失败会导致糖尿病(DM)，可以是I型糖尿病(T1D，占确诊糖尿病的5%)，以前称为青少年糖尿病或胰岛素依赖型糖尿病，或II型糖尿病(T2D，占确诊糖尿病的95%)，以前称为成人发病型糖尿病或胰岛素非依赖型糖尿病 (4)。T1D是原发性β细胞胰岛素分泌不足，而T2D是功能性胰岛素缺乏，主要由靶细胞的胰岛素抵抗引起。胰岛素基因突变是新生儿糖尿病的一个原因 (2, 5, 6)。随着时间的推移，糖尿病发病率越来越高，2011年美国就有7900万成年人(占总人口的8%)。发病率随着年龄(27%的成年人>65岁)和肥胖而增加，并随种族背景而变化。糖尿病是美国第七大死因 (2, 4)。并发症包括心脏病、中风、高血压、失明、肾病和神经病变 (4)。

胰岛素是以110个氨基酸(aa)的前原蛋白形式合成。24个aa信号序列被切割形成86个 aa的胰岛素原肽，该肽经过进一步蛋白水解生成30个aa的B链 (前原蛋白的aa 25-54)，21个aa的A链 (aa 90-110) 和34个aa的介导C肽 (1, 2)。成熟人胰岛素是二硫键连接的A和B链的异二聚体，与猪和犬胰岛素序列同源性为98% (50/51 aa)，与牛胰岛素序列同源性为94% (48/51 aa)，与小鼠、大鼠、猫和羊胰岛素序列同源性为92% (47/51 aa)。胰岛素以与锌离子配位的六聚体形式储存在β细胞中 (2)。胰岛素以无锌单体的形式被释放到肝门静脉，从而在肝脏中达到最高浓度 (2, 7)。基础水平持续分泌，更高的分泌受到食物摄入的刺激 (7)。对于需要胰岛素替代或补充的糖尿病患者(26%的糖尿病患者)，通过注射或植入泵将天然胰岛素或合成类似物输送至皮下 (4, 7)。

胰岛素通过胰岛素受体(Ins R)发挥作用，胰岛素受体是一种酪氨酸激酶，在所有细胞上都有不同数量的存在。胰岛素通过结合和交联两个受体分子形成三聚体受体/配体复合物来启动下游信号传导 (2, 3, 8)。信号通过调节细胞膜葡萄糖转运体的出现促进细胞对葡萄糖的摄取。Ins R存在A和B两个亚型，它们可能与IGF-I受体同二聚或异二聚 (3, 6)。所有受体组合结合胰岛素、IGF-I或IGF-II，但亲和力不同 (3, 6)。该系统允许根据胰岛素、IGF-I和IGF-II的浓度以及细胞表面受体亚单位的表达对胰岛素介导的信号通路激活进行微调 (6)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人Insulin抗体包被于微孔板上，样品和标准品中的人Insulin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Insulin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-C（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 (pmol/L)	383.6	178.4	69.6	386.8	178.4	61.6
标准差	20.4	4.4	6.4	19.6	4.0	4.4
CV%	5.3	2.5	9.3	5.1	2.1	7.0

B. 回收率

在细胞培养上清样本中掺入检测范围内不同水平的人Insulin，测定其回收率。回收率范围在88.2-117.1%，平均回收率在106.1%。

C. 灵敏度

人Insulin的最低可测剂量（MDD）一般小于0.14 pmol/L。

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生产的酿酒酵母（*S.cerevisiae*）表达的高纯度重组人Insulin蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人Insulin，然后用标准品稀释液-C（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	99.3	86.6-118.5
1:4	104.6	92.3-123.8
1:8	101.5	89.3-113.7
1:16	104.4	95.4-113.4

F. 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。

Recombinant human	
C-Peptide	Relaxin-1
IGF-I	Relaxin-2
	Relaxin-3

该Valukine™还识别天然的犬和猪胰岛素。

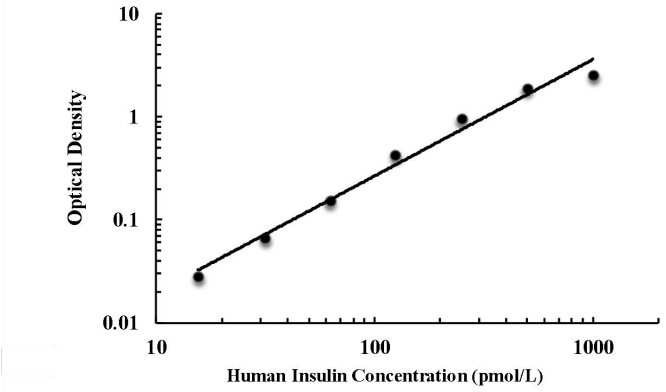
重组人(rh)胰岛素受体和重组人IGF-II在本实验中没有交叉反应，但在浓度分别大于1.56和3.13 ng/mL时产生干扰。

含有25.0 ng/mL重组人胰岛素原的样品检测值为922 pg/mL (交叉反应率为3.7%)。

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。



pmol/L	OD	Average	Corrected
0	0.009 0.009	0.009	-
15.6	0.035 0.040	0.037	0.028
31.3	0.077 0.074	0.076	0.067
62.5	0.151 0.170	0.161	0.152
125	0.435 0.441	0.438	0.429
250	0.955 0.979	0.967	0.958
500	1.951 1.802	1.877	1.868
1000	2.503 2.543	2.523	2.514

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Insulin Microplate	包被抗人 Insulin 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human Insulin Standard	人 Insulin 标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Human Insulin Detection Antibody	生物素化的抗人 Insulin 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Calibrator Diluent-C (1×)	标准品稀释液-C（1×）用于稀释标准品和样本	2 瓶
Detection Antibody Diluent-C Concentrate (4×)	浓缩的检测抗体稀释液-C（4×）用于稀释检测抗体	1 瓶
Streptavidin-HRP B (40×)	40×浓缩的链霉亲和素标记的 HRP	1 瓶
Reagent Diluent Concentrate (10×)	浓缩的试剂稀释液（10×）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="8">已打开，稀释或重溶的试剂</td><td>链霉亲和素-HRP B</td><td rowspan="4">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>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-C（1×）</td><td>2-8℃储存，最多 30 天*</td></tr>
<tr><td>检测抗体稀释液-C（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-C，多余的丢弃</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，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-C（1×）稀释。

B. 样本准备工作

细胞上清样本建议用标准品稀释液-C（1×）4倍稀释后进行检测，例如：50 µL样本+150 µL**标准品稀释液-C（1×）**。最佳稀释度应由最终用户确定。

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Insulin，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浓缩洗涤液用去离子水或蒸馏水稀释配制成 500 mL工作浓度的洗涤液（1×）。

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

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×）。

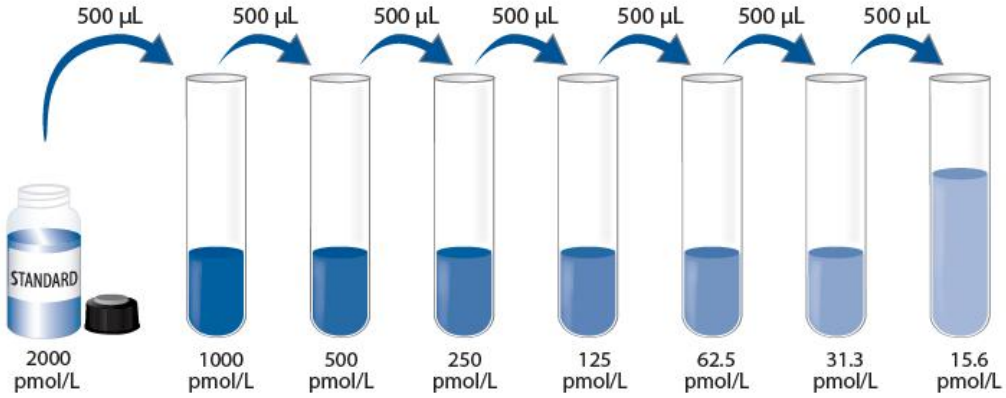
检测抗体（1×）：开盖前请瞬时离心。参考检测抗体瓶标签重溶冻干粉，制备检测抗体（100×）。轻轻震摇至少15分钟，其充分溶解。如有需要分装保存。再用检测抗体稀释液-C（1×）稀释至检测抗体（1×），至少在使用前15分钟准备。

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

人Insulin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2000 pmol/L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500 µL**标准品稀释液-C（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。1000 pmol/L管作标准曲线最高点，**标准品稀释液-C（1×）**可用作标准品零点（0 pmol/L）。



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，TMB底物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 Insulin，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 500±50 rpm 水平振荡孵育 1 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 用检测抗体稀释液-C（1×）稀释好的检测抗体（1×）。用封板膜封住反应孔，**室温 500±50 rpm 水平振荡孵育 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 B 工作液。用封板膜封住反应孔，**室温 500±50 rpm 水平振荡孵育 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 500±50 rpm 水平振荡孵育 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；
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 Insulin 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