PRODUCT INFORMATION & MANUAL

Human Leptin Valukine™ ELISA

Catalog Number: VAL211

For the quantitative determination of natural and recombinant human Leptin 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 202411.1

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I. BACKGROUND

Human Leptin (gene name OB) is a 16 kDa, 146 amino acid (aa) residue, non-glycosylated polypeptide that regulates adipose tissue mass and energy balance (1-6). The name Leptin is derived from the Greek (leptos, or "thin") because of its ability to reduce fat stores (7). In mice (ob/ob) and humans, inactivating mutations of the OB gene can cause obesity (1-6). Mature human Leptin shares 87% and 84% aa identity with mouse and rat Leptin, respectively (1, 8). Human Leptin is active in both the mouse and rat systems (9, 10). Leptin is expressed almost exclusively by adipocytes and its production is influenced by hormones, cytokines and nutrients (5, 8, 11). For example, Leptin expression is enhanced by insulin and glucocorticoids, which are associated with positive energy balance, while catecholamines decrease Leptin production during negative energy balance (5). It circulates in the plasma, crosses the blood-brain barrier, and is present in human breast milk (3-6, 12).

The human Leptin receptor (designated ObR or LEPR) is a 150 kDa, 1144 aa residue, type I transmembrane glycoprotein of the IL-6 receptor family of Class I cytokine receptors (13, 14). The gene for ObR undergoes considerable splicing, forming variants a-d with cytoplasmic domains of variable length, plus the potentially soluble form ObRe (14, 15). The long form, ObRb (formerly OB RL), is expressed mainly in the hypothalamic arcuate nucleus and is essential for signal transduction (6, 16, 17). Of the short forms, ObRa is ubiquitous, and ObRa, ObRc, and ObRd are all thought to mediate Leptin binding and endocytosis, but not signal transduction (16). Upon binding of Leptin dimers, ObRb dimers may form signaling tetramers with shorter forms (16). Mutations of ObRb can cause obese phenotypes in both the mouse and rat. The mouse mutation (db/db for diabetes) occurs in the cytoplasmic domain, while the rat mutation (fa/fa for fatty) occurs in the extracellular domain of the receptor (18, 19). In a concentration-dependent manner, Leptin signaling can have diverse effects, causing neurons that express pro-opiomelanocortin (POMC) peptides to reduce food intake, and neurons that express neuropeptide Y and agouti-related protein (NpY and AgRP) to increase food intake (4, 6).

Leptin is fundamentally a "starvation signal" that, when low, prompts increased appetite and decreased energy expenditure (4, 6, 10). Adipocytes increase Leptin expression as cell size increases, which should result in depressed appetite and

increased energy expenditure (5). However, obese humans are often resistant to these effects of Leptin (3). Leptin resistance is in part due to saturation of the blood-brain transporter, which is influenced by high circulating triglycerides, and in part due to decreased cellular response to Leptin (6). Rarely, obese humans are genetically Leptin-deficient (3-6). Leptin deficiency also influences the immune system, depressing Th1 responses and causing increased frequency of infections (4). Leptin also regulates puberty, blocking the onset of puberty, or of menses if Leptin deficiency exists due to excessive thinness, such as results from starvation, extreme exercise-induced weight loss, anorexia or cancer-induced cachexia (3, 4).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Leptin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any human Leptin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for human Leptin is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, TMB substrate (Chromogenic agent) is added to the wells and color develops in proportion to the amount of human Leptin 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, human serum and human 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 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 forty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	64.5	146	621	65.7	146	581
Standard Deviation	2.14	4.32	20.0	3.56	6.17	20.6
CV%	3.3	3.0	3.2	5.4	4.2	3.5

B. RECOVERY

The recovery of human Leptin spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture media (n=5)	98	94-102
Human Serum* (n=5)	95	89-109
Human EDTA plasma* (n=5)	99	85-112
Human Heparin plasma* (n=5)	90	81-100
Human Citrate plasma* (n=5)	95	87-105

*Samples were initially diluted prior to assay as directed in the Sample Preparation section.

C. SENSITIVITY

The minimum detectable dose (MDD) of human Leptin is typically less than 7.8 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 Leptin produced at R&D Systems.

The NIBSC/WHO Leptin (human rDNA derived) WHO International Standard 97/594 was evaluated in this kit. The dose response curve of the NIBSC standard 97/594 parallels the Valukine standard curve. To convert sample values obtained with the Valukine Human Leptin kit to approximate NIBSC 97/594 Units, use the equation below.

NIBSC/WHO (97/594) approximate value (mIU/mL) = 0.849 × Valukine Human Leptin value (pg/mL)

Note: *Based on data generated in October 2020.*

E. LINEARITY

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

Dilution		Cell culture media (n=5)	Human Serum* (n=5)	Human EDTA plasma* (n=5)	Human Heparin plasma* (n=5)	Human Citrate plasma* (n=5)
1:2	Average % of Expected	105	99	99	99	98
	Range (%)	103-107	99-101	97-102	96-104	96-99
1:4	Average % of Expected	109	97	95	97	96
	Range (%)	106-114	94-102	94-99	93-100	93-99
1:8	Average % of Expected	109	92	92	94	93
	Range (%)	107-115	89-95	90-94	90-97	89-97
1:16	Average % of Expected	109	92	91	96	93
	Range (%)	106-113	87-97	86-94	90-100	89-96

*Samples were diluted prior to assay.

F. SAMPLE VALUES

Human Serum - Samples from apparently healthy volunteers were evaluated for the presence of human Leptin in this assay. No medical histories were available for the donors used in this study.

Sample Type	Range (pg/mL)	Mean (pg/mL)
Human Male Serum (n = 16)	2205-11,149	4760
Human Female Serum (n = 36)	3877-77,273	20,676

Five additional human male serum samples fell below the lowest standard, 15.6 pg/mL, when diluted 100-fold.

Note: *Values in human EDTA and human heparin plasma have been found to be comparable to paired human serum samples. Values in human citrate plasma have been found to be slightly decreased compared to paired human serum, human EDTA or human heparin plasma samples.*

Cell Culture Supernates:

Human peripheral blood mononuclear cells (5 × 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the culture supernates were removed on days 1 and 5 and assayed for levels of human Leptin.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	ND
Stimulated	152	75.9

ND=Non-detectable

BeWo human choriocarcinoma cells (1 × 10^6 cells/mL) were cultured in F-12 media supplemented with 15% fetal bovine serum. The cells were cultured unstimulated or stimulated with 2 µM forskolin and 20 µM forskolin. Aliquots of the cell culture supernates were removed on days 1, 2, and 3 and assayed for levels of human Leptin.

Condition	Day 1 (pg/mL)	Day 2 (pg/mL)	Day 3 (pg/mL)
Unstimulated*	849	1549	1667
2 µM forskolin*	1231	1699	2054
20 µM forskolin*	1137	1725	2747

*Samples were diluted 20-fold prior to assay.

G. SPECIFICITY

This assay recognizes natural and recombinant human Leptin.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human Leptin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human：			Recombinant mouse:
ANG	IL-2 Rα	PTN	GM-CSF
AR	IL-3	RANTES	IL-1α
CNTF	IL-3 Rα	SCF	IL-1β
CRP	IL-4	SLPI	IL-3
β-ECGF	IL-4 R	TGF-α	IL-4
EGF	IL-5	TGF-β1	IL-5
Epo	IL-5 Rβ	TGF-β3	IL-5 Rα
FGF acidic	IL-6	TGF-βRII	IL-6
FGF basic	IL-6 R	TNF-α	IL-7
FGF-4	IL-7	TNF-β	IL-9
FGF-5	IL-8	TNF RI	IL-10
FGF-6	IL-9	TNF RII	IL-13
G-CSF	IL-10	VEGF	Leptin

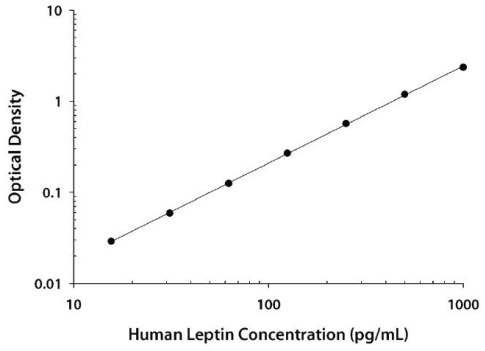
GM-CSF	IL-11		LIF
gp130	IL-12		MIP-1α
GROα	IL-13		MIP-1β
GROβ	KGF		SCF
GROγ	LAP (TGF-β1)		TNF-α
HB-EGF	LIF		**Recombinant amphibian:**
HGF	M-CSF		TGF-β5
IFN-γ	MCP-1		**Natural proteins:**
IGF-I	MIP-1α		bovine FGF acidic
IGF-II	MIP-1β		bovine FGF basic
IL-1α	β-NGF		human PDGF
IL-1β	OSM		porcine PDGF
IL-1ra	PD-ECGF		human TGF-β1
IL-1 RI	PDGF-AA		porcine TGF-β1
IL-1 RII	PDGF-AB		porcine TGF-β2
IL-2	PDGF-BB		

Recombinant human Leptin R/Fc chimera and recombinant mouse Leptin R/Fc chimera do not cross-react, but do interfere at concentrations ≥ 0.78 ng/mL in this assay.

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)	O.D.	Average	Corrected
0	0.015 0.015	0.015	—
15.6	0.044 0.044	0.044	0.029
31.3	0.073 0.075	0.074	0.059
62.5	0.136 0.144	0.140	0.125
125	0.282 0.285	0.284	0.269
250	0.581 0.588	0.584	0.569
500	1.195 1.211	1.203	1.188
1000	2.339 2.415	2.377	2.362

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Leptin Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against human Leptin.	1 plate
Human Leptin Conjugate	Solution of antibody against human Leptin conjugated to horseradish peroxidase.	1 vial
Human Leptin Standard	Recombinant human Leptin in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Assay Diluent RD1-19	A buffered protein base.	1 vial
Calibrator Diluent Concentrate (5×)/ RD5P	A 5× concentrated buffered protein base used to dilute standard and samples.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant.	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution.	2 vials
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="6">Opened/
Reconstituted
Reagents</td><td>Wash Buffer (1×)</td><td rowspan="4">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Standard</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Assay Diluent
RD1-19</td><td>May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Calibrator Diluent
Concentrate (5×)/
RD5P</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>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.
- 100 mL 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 eye, hand, face, and clothing protection when using this material.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

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×).

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 × 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 Calibrator Diluent (1×).

Note: *Grossly lipemic samples are not suitable for use in this assay.*

B. SAMPLE PREPARATION

Human serum and plasma samples recommend a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Note: *Bring all reagents to room temperature before use.*

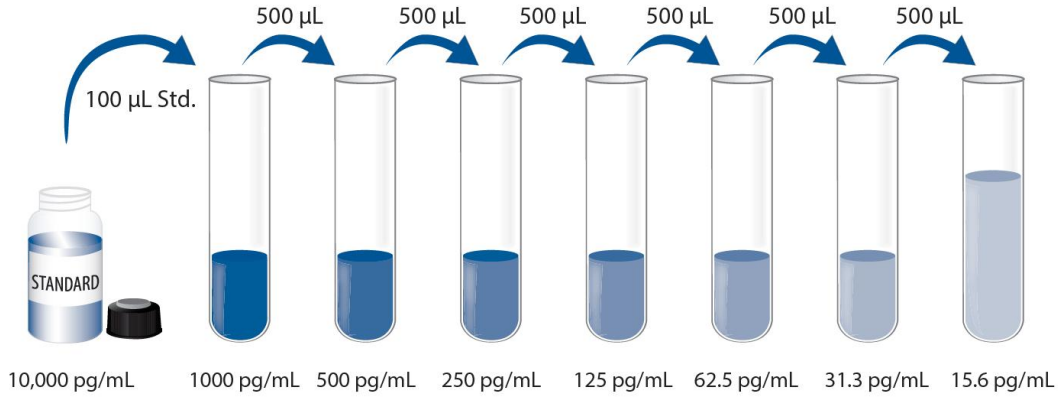
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

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

Human Leptin Standard - Refer to the vial label for the reconstitution volume.* Reconstitute the Human Leptin Standard with deionized or distilled water. This reconstitution produces a stock solution of 10000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

*If you have any questions, please seek help from our Technical Support.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent (1×) into the 1000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard stock serves as the high standard (1000 pg/mL). 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 other reagents and samples to room temperature before use. It is recommended that all standards and samples 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 Assay Diluent RD1-19 to each well.
4. 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.
5. 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.
6. Add 200 µL of Human Leptin Conjugate to each well. Cover with a new adhesive strip. **Incubate for 1 hour at room temperature.**
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. 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.

11. 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 log/log 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 a log/log graph. The data may be linearized by plotting the log of the human Leptin concentrations versus the log of the O.D. on a linear scale and the best fit line can be determined by regression analysis.

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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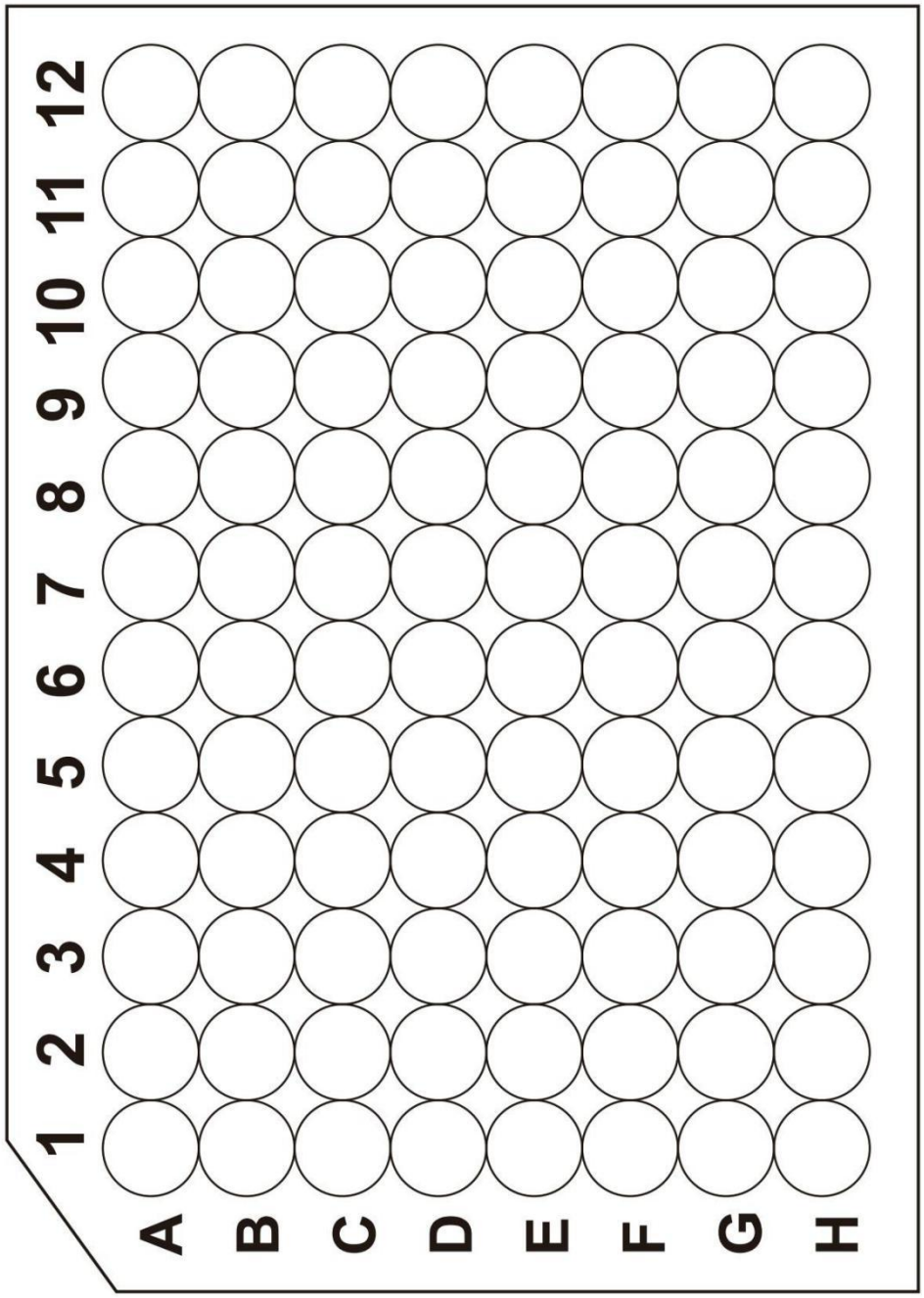
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 Leptin Valukine™ ELISA 试剂盒

目录号：VAL211

适用于定量检测天然和重组人 Leptin 的浓度

科研专用，不可用于临床诊断

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版本号 202411.1

目录

I. 背景

人瘦素（Leptin,基因名 OB）是一种 16 kDa、146 个氨基酸（amino acid, aa）残基、非糖基化的多肽，可调节脂肪组织的质量和能量平衡（1-6）。Leptin 的名称源自希腊语（leptos，或“thin”），因为它能够减少脂肪储存（7）。在小鼠（ob/ob）和人类中，OB 基因的失活突变可导致肥胖（1-6）。成熟的人 Leptin 与小鼠和大鼠 Leptin 的 aa 相同度分别为 87%和 84%（1,8）。人 Leptin 在小鼠和大鼠系统中都具有活性（9,10）。Leptin 几乎完全由脂肪细胞表达，其产生受激素、细胞因子和营养物质的影响（5,8,11）。例如，胰岛素和糖皮质激素会增强瘦素的表达，这与正能量平衡有关，而儿茶酚胺则会在负能量平衡时减少瘦素的产生（5）。它在血浆中循环，穿过血脑屏障，并存在于母乳中（3-6,12）。

人瘦素受体（定名为 ObR 或 LEPR）是一种 150 kDa、1144 aa 残基的 I 型跨膜糖蛋白，属于 IL-6 受体家族的 I 类细胞因子受体（13,14）。ObR 的基因经过大量剪接，形成具有不同长度细胞质结构域的 a-d 变体，以及可能可溶的 ObRe（14,15）。长型 ObRb（前身为 OB RL）主要在下丘脑弓状核中表达，对信号转导至关重要（6,16,17）。在短形式中，ObRa 无处不在，ObRa、ObRc 和 ObRd 都被认为介导 Leptin 的结合和内吞，但不介导信号转导（16）。与 Leptin 二聚体结合后，ObRb 二聚体可能会以较短的形式形成信号四聚体（16）。ObRb 基因突变可导致小鼠和大鼠出现肥胖表型。小鼠的突变（db/db 糖尿病型）发生在细胞质结构域，而大鼠的突变（fa/fa 肥胖型）发生在受体的细胞外结构域（18,19）。Leptin 信号以浓度依赖的方式产生多种影响，可导致表达前绒毛膜促皮质素（pro-opiomelanocortin，POMC）肽的神经元减少食物摄入量，而表达神经肽 Y 和激动相关蛋白（NpY 和 AgRP）的神经元增加食物摄入量（4,6）。

Leptin 从根本上说是一种 “饥饿信号”，当其含量较低时，会促使食欲增加和能量消耗减少（4,6,10）。随着细胞体积的增大，脂肪细胞会增加 Leptin 的表达，从而抑制食欲，增加能量消耗（5）。然而，肥胖的人往往对瘦素的这些作用有抵抗力（3）。Leptin 抵抗一部分是由于血脑转运体饱和，而血脑转运体受到高甘油三酯循环的影响，一部分原因是细胞对 Leptin 的反应降低（6）。罕见的是，肥胖的人在基因上缺乏 Leptin（3-6）。Leptin 缺乏还会影响免疫系统，抑制 Th1 反应，导致感染频率增加(4)。Leptin 还能调节青春期，阻止青春期的到来，如果因如饥饿、剧烈运动导致的体重减轻、厌食症或癌症引起的恶病质等原因而过度消瘦会造成瘦素缺乏，瘦素还能阻止月经的到来（3,4）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗人Leptin抗体包被于微孔板上，样品和标准品中的人Leptin会与固定在板上的抗体结合，游离的成分被洗去；加入辣根过氧化酶标记的抗人Leptin检测抗体进行孵育。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，人血清和人血浆；
- 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×）稀释并重复测定。
- 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，在不同板间分别检测40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)	64.5	146	621	65.7	146	581
标准差	2.14	4.32	20.0	3.56	6.17	20.6
CV%	3.3	3.0	3.2	5.4	4.2	3.5

B. 回收率

不同类型样本中掺入检测范围内不同水平的人Leptin，测定其回收率。

样本类型	平均回收率 (%)	范围 (%)
细胞培养基 (n=5)	98	94-102
人血清* (n=5)	95	89-109
人 EDTA 血浆* (n=5)	99	85-112
人肝素血浆* (n=5)	90	81-100
人柠檬酸血浆* (n=5)	95	87-105

*样品在分析前按照样品制备部分的指示进行稀释。

C. 灵敏度

人 Leptin 的最小可检测剂量（MDD）通常小于 7.8 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

该免疫测定法以R&D Systems生产的高纯度的大肠杆菌表达的重组人Leptin校正。

该试剂盒评估了NIBSC/WHO Leptin（人源rDNA）WHO国际标准97/594。NIBSC标准97/594的剂量反应曲线与Valukine标准曲线相似。要将使用 Valukine人Leptin试剂盒获得的样本值转换为近似NIBSC 97/594单位，请使用以下方程式。

NIBSC/WHO（97/594）近似值（mIU/mL）= 0.849 × Valukine 人Leptin值（pg/mL）。

注：*本数据基于2020年10月。*

E. 线性

不同的样本中含有或掺入高浓度的人Leptin，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		细胞培养基 (n=5)	人血清* (n=5)	人EDTA血浆* (n=5)	人肝素血浆* (n=5)	人柠檬酸血浆* (n=5)
1:2	平均值/期待值（%）	105	99	99	99	98
	范围 (%)	103-107	99-101	97-102	96-104	96-99
1:4	平均值/期待值（%）	109	97	95	97	96
	范围 (%)	106-114	94-102	94-99	93-100	93-99
1:8	平均值/期待值（%）	109	92	92	94	93
	范围 (%)	107-115	89-95	90-94	90-97	89-97
1:16	平均值/期待值（%）	109	92	91	96	93
	范围 (%)	106-113	87-97	86-94	90-100	89-96

*样品在检测前进行了稀释。

F. 样本预值

人血清 – 在本试验中评估了来自表面健康志愿者的样本中是否存在人 Leptin。本研究中使用的供体没有病史。

样本类型	范围 (pg/mL)	平均值 (pg/mL)
人男性血清 (n=16)	2205-11,149	4760
人女性血清 (n=36)	3877-77,273	20,676

另外 5 份人男性血清样品稀释100倍后低于最低标准品，15.6 pg/mL。

注：*已发现人 EDTA和人肝素血浆中的值与配对的人血清样品相当。研究发现，与配对的人血清、人EDTA或人肝素血浆样品相比，人柠檬酸盐血浆中的值略有降低。*

细胞培养上清液–人外周血单核细胞（5×10⁶ cells/mL）在RPMI培养基中培养，该培养基补充了5%胎牛血清、50 μMβ-巯基乙醇、2 mM L-谷氨酰胺、100 U/mL青霉素和100 μg/mL链霉素硫酸盐。细胞在不刺激或用10 μg/mL PHA刺激的情况下培养。在第1天和第5天取培养上清液，测定人Leptin水平。

条件	1天 (pg/mL)	5天 (pg/mL)
未刺激	ND	ND
刺激	152	75.9

ND=未检出

BeWo 人绒毛膜细胞（1×10⁶ cells/mL）在添加了 15%胎牛血清的 F-12 培养基中培养。细胞未经刺激或用 2 μM Forskolin 和 20 μM Forskolin 刺激进行培养。在第 1 天、第 2 天和第 3 天取出细胞培养上清液，检测人 Leptin 水平。

条件	1天 (pg/mL)	2天 (pg/mL)	3天 (pg/mL)
未刺激*	849	1549	1667
2 μM forskolin刺激*	1231	1699	2054
20 μM forskolin刺激*	1137	1725	2747

*样品在测定前稀释20倍。

G. 特异性

检测方法识别天然和重组人Leptin。

以下列出的因子在标准品稀释液（1×）中以50 ng/mL的浓度制备，并进行交叉反应性测定。以下列出的因子在中值范围重组人Leptin对照品中以50 ng/mL的浓度制备，并进行干扰测定。未观察到明显的交叉反应或干扰。

Recombinant human：			**Recombinant mouse:**
ANG	IL-2 Rα	PTN	GM-CSF
AR	IL-3	RANTES	IL-1α
CNTF	IL-3 Rα	SCF	IL-1β
CRP	IL-4	SLPI	IL-3
β-ECGF	IL-4 R	TGF-α	IL-4
EGF	IL-5	TGF-β1	IL-5
Epo	IL-5 Rβ	TGF-β3	IL-5 Rα
FGF acidic	IL-6	TGF-βRII	IL-6
FGF basic	IL-6 R	TNF-α	IL-7
FGF-4	IL-7	TNF-β	IL-9
FGF-5	IL-8	TNF RI	IL-10
FGF-6	IL-9	TNF RII	IL-13
G-CSF	IL-10	VEGF	Leptin
GM-CSF	IL-11		LIF
gp130	IL-12		MIP-1α
GROα	IL-13		MIP-1β
GROβ	KGF		SCF
GROγ	LAP (TGF-β1)		TNF-α
HB-EGF	LIF		**Recombinant amphibian:**
HGF	M-CSF		TGF-β5
IFN-γ	MCP-1		**Natural proteins:**
IGF-I	MIP-1α		bovine FGF acidic

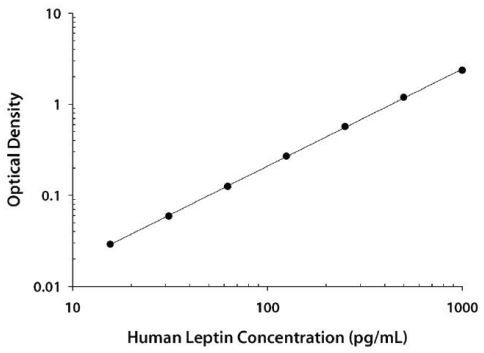
IGF-II	MIP-1β		bovine FGF basic
IL-1α	β-NGF		human PDGF
IL-1β	OSM		porcine PDGF
IL-1ra	PD-ECGF		human TGF-β1
IL-1 RI	PDGF-AA		porcine TGF-β1
IL-1 RII	PDGF-AB		porcine TGF-β2
IL-2	PDGF-BB		

重组人Leptin R/Fc嵌合体和重组小鼠Leptin R/Fc嵌合体在此检测中不发生交叉反应，但浓度≥0.78 ng/mL时会产生干扰。

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)	O.D.	Average	Corrected
0	0.015 0.015	0.015	—
15.6	0.044 0.044	0.044	0.029
31.3	0.073 0.075	0.074	0.059
62.5	0.136 0.144	0.140	0.125
125	0.282 0.285	0.284	0.269
250	0.581 0.588	0.584	0.569
500	1.195 1.211	1.203	1.188
1000	2.339 2.415	2.377	2.362

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Leptin Microplate	包被抗人Leptin抗体的96孔聚苯乙烯板，8孔×12条	1块板
Human Leptin Conjugate	酶标检测抗人Leptin抗体	1瓶
Human Leptin Standard	重组人Leptin标准品（冻干），参考瓶身标签进行重溶	1瓶
Assay Diluent RD1-19	检测液	1瓶
Calibrator Diluent Concentrate (5×)/ RD5P	浓缩的标准品稀释液（5×），用于稀释标准品和样品	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	2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°C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>洗涤液（1×）</td><td rowspan="4">2-8°C储存，最多30天*</td></tr>
<tr><td>终止液</td></tr>
<tr><td>酶标检测抗体</td></tr>
<tr><td>TMB底物溶液</td></tr>
<tr><td>标准品</td><td>2-8°C储存，最多30天*</td></tr>
<tr><td>检测液RD1-19</td><td>2-8°C储存，最多30天*</td></tr>
<tr><td>浓缩的标准品稀释液（5×）/ RD5P</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>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 °C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
- 100 mL和500 mL量筒

D. 注意事项

- 试剂盒中的一些组分含有防腐剂，可能引起皮肤过敏反应，避免吸入。
- 试剂盒中的终止液是酸性溶液，使用时请做好眼睛、手、面部及衣服的防护。

VI. 实验前准备

A. 样品收集及储存

以下列出的样品收集和储存条件仅作为一般指南。样品稳定性尚未评估。

细胞培养上清液 - 通过离心去除颗粒物，立即或等分进行检测，并将样品储存在≤ -20°C的温度下，避免反复冻融。样品可能需要用标准品稀释液（1×）稀释。

血清 - 使用血清分离管(SST)，让样本在室温下凝固30分钟，然后在1000 × g 的离心力下离心15分钟。分离血清并立即进行检测，或分装并储存在≤ -20 °C。避免反复冻融循环。样本可能需要用标准品稀释液（1×）稀释。

血浆 - 使用EDTA、肝素或柠檬酸盐作为抗凝剂收集血浆。在采样后30分钟内，以1000 × g的离心力离心15分钟。立即检测或分装并储存在≤ -20 °C。避免反复冻融。样品可能需要用标准品稀释液（1×）稀释。

注：*严重脂血样本不得用于本试验。*

B. 样品准备

人血清和血浆样品建议进行 100 倍稀释。建议的 100 倍稀释量为：10 µL 的样本+990 µL 的标准品稀释液（1×）。最佳稀释倍数应由用户自行确定。

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（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×）。

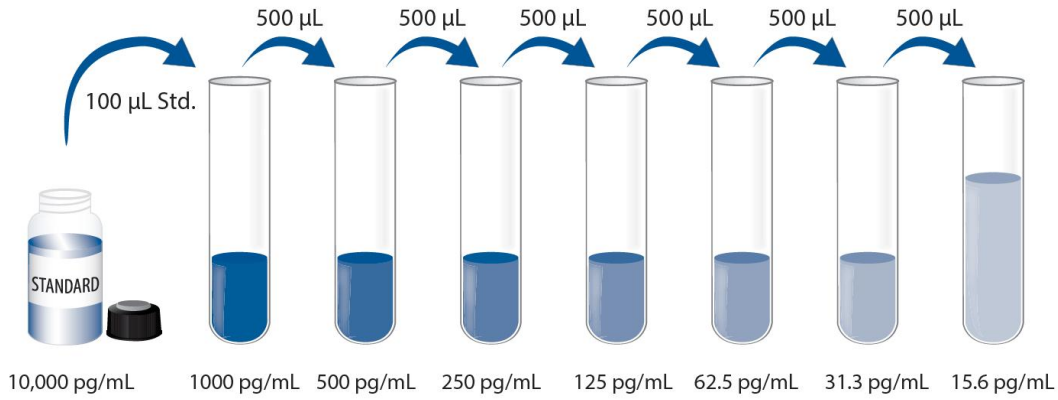
标准品稀释液（1×）：使用去离子水或蒸馏水制备标准品稀释液（1×）。

人Leptin标准品：重溶体积请参考瓶身标签*，用去离子水或蒸馏水复溶人Leptin标准品，得到浓度为10000 pg/mL标准品储备液。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。

使用聚丙烯管。将900 µL标准品稀释液（1×）移入1000 pg/mL管中，剩余管中各加入500 µL。使用标准品储备液制备以下稀释系列（如下图所示）。在下次转移之前，将每管彻底混合。未稀释的标准品储备液作为最高标准品（1000 pg/mL）。标准品稀释液

（1×）作为标准品零点（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，将所有其他试剂和样品带至室温。建议对所有标准品和样品进行复孔检测。

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. 向每个孔中加入100 µL的检测液RD1-19。
4. 向每个孔中加入100 µL的标准品和制备好的样品，用封板膜封住反应孔，**在室温下孵育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。
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。
6. 在每个微孔内加入200 µL人Leptin酶标检测抗体。用封板膜封住反应孔，**室温孵育1小时**。
7. 重复第5步洗板操作。
8. 在每个孔内加入200 µL TMB底物溶液，**室温孵育30分钟。注意避光**。
9. 在每个孔内加入50 µL终止液，请轻拍微孔板，使溶液混合均匀。
10. 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。
11. 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log/log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log/log图上的点绘制最佳拟合曲线。数据可以通过绘制人Leptin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。

    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