PRODUCT INFORMATION & MANUAL

Mouse Leptin Valukine™ ELISA

Catalog Number: VAL618

For the quantitative determination of natural and recombinant mouse 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 202407.4

TABLE OF CONTENTS

I. BACKGROUND

Mouse Leptin is a protein product of the mouse obese gene (1, 2). Mutant mice that lack functional Leptin have been found to be obese, diabetic, infertile and to have reduced activity, metabolism and body temperature. cDNA clones encoding Leptin have been isolated from human, simian, mouse and rat cells. Mature mouse Leptin shares approximately 96% and 84% amino acid (aa) sequence identity with the rat and human proteins, respectively. Mouse Leptin cDNA encodes a 167 aa residue protein with a 21 aa residue signal sequence that is cleaved to yield the 146 aa residue mature protein. The expression of Leptin mRNA has been shown to be restricted to adipose tissues and placenta (2).

A high-affinity receptor for Leptin (OB-R) with homology to gp130, G-CSF receptor, and LIF receptor has been cloned (3). Multiple isoforms of OB-R, including a long form (OB-R_L) with a large cytoplasmic domain capable of signal transduction, and several receptor isoforms with short cytoplasmic domains (OB-R_S) lacking signal transducing capabilities, have been identified (4-6). An OB-R transcript lacking a transmembrane domain and potentially encoding a soluble form of the receptor has also been described (7). OB-R_L transcripts were reported to be expressed predominantly in regions of the hypothalamus previously thought to be important in body weight regulation. Expression of OB-R_S transcripts have been found in multiple tissues, including the choroid plexus, lung, kidney and primitive hematopoietic cell populations (2). OB-R has been shown to be encoded by the mouse diabetes (db) and rat fatty (fa) genes (8). Rodents homozygous for the db or fa mutations have long been known to exhibit an obesity phenotype almost identical to the phenotype of ob/ob mice (9).

High Leptin levels (ng/mL) have been detected in mouse, rat and human serum or plasma. Circulating levels of Leptin have been shown to be regulated in response to a variety of stimuli including food intake, insulin, glucocorticoids, cytokines, and reproductive events (2, 10-14). The majority of Leptin in serum was reported to be bound to multiple Leptin-binding proteins (15).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse Leptin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any mouse Leptin present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for mouse Leptin 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, TMB substrate solution (Chromogenic agent) is added to the wells and color develops in proportion to the amount of 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 and mouse 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 Reagent 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)	180.4	1248	4429	186.2	1271.8	4464.1
Standard Deviation	4.7	33.3	113.9	8.2	54.2	130.6
CV%	2.6	2.7	2.6	4.4	4.3	2.9

B. RECOVERY

The recovery of mouse Leptin spiked to different levels throughout the range of the assay in different samples was evaluated. The recovery ranged from 85.2 to 105.7% with an average of 96.4%.

C. SENSITIVITY

The minimum detectable dose (MDD) of mouse Leptin is typically less than 5.35 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. CALIBRATION

This immunoassay is calibrated against highly purified *E.coli*-expressed recombinant mouse Leptin produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with

high concentrations of mouse Leptin and diluted with Reagent Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	100.0	93.6 - 106.1
1:4	99.1	94.6 - 104.0
1:8	94.7	89.7 - 100.8
1:16	92.5	87.5 - 97.2

F. SPECIFICITY

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

Recombinant mouse	Recombinant human
Leptin R	Leptin R

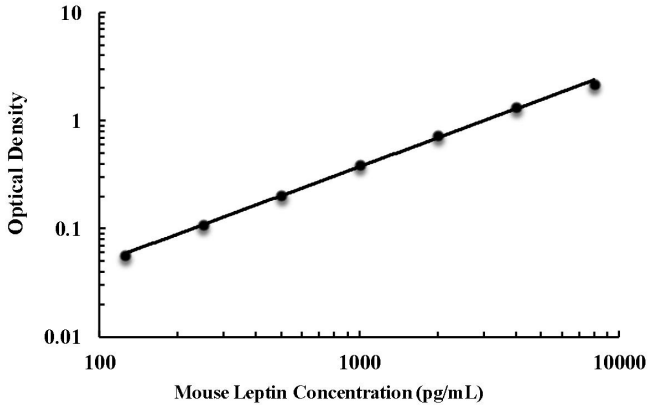
A sample containing 50 ng/mL of recombinant human Leptin reads as 94 pg/mL (0.19% cross-reactivity).

A sample containing 1.6 ng/mL of recombinant rat Leptin reads as 651 pg/mL (42% 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	0.036 0.034	0.035	-
125	0.093 0.090	0.092	0.057
250	0.142 0.144	0.143	0.108
500	0.238 0.235	0.236	0.201
1000	0.434 0.415	0.424	0.389
2000	0.782 0.738	0.760	0.725
4000	1.345 1.408	1.376	1.341
8000	2.194 2.233	2.213	2.178

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse Leptin Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse Leptin.	1 plate
Mouse Leptin Standard	Recombinant mouse Leptin in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Mouse Leptin Detection Antibody	Biotinylated mouse Leptin antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Reagent Diluent Concentrate 3 (5×)	A 5× concentrated buffered diluent used to prepare Reagent Diluent (1×) to dilute standard, samples, Detection Antibody and HRP.	1 vial
PBS Concentrate (20×)	Used to dilute Reagent Diluent (1×)	1 vial
Streptavidin-HRP B (40×)	40× Streptavidin conjugated to horseradish peroxidase.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB ELISA Substrate Solution/ 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="7">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>Wash Buffer (1×)</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>PBS Concentrate (20×)</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 3 (5×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*</td></tr>
</table>

* Provided this is within the expiration date of the kit.

C. OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.

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 Reagent Diluent (1×).

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Reagent Diluent (1×).

B. SAMPLE PREPARATION

Mouse serum samples recommend a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Reagent 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.

PBS (1×) - Use deionized or distilled water to prepare PBS (1×). For example, add 3 mL of PBS Concentrate (20×) to 57 mL deionized or distilled water to prepare 60 mL PBS (1×).

Reagent Diluent (1×) - Use PBS (1×) to prepare Reagent Diluent (1×). For example, add 12 mL of Reagent Diluent Concentrate 3 (5×) to 48 mL of PBS (1×) to prepare 60 mL of 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 Reagent Diluent (1×). Prepare at least 15 minutes prior to use.

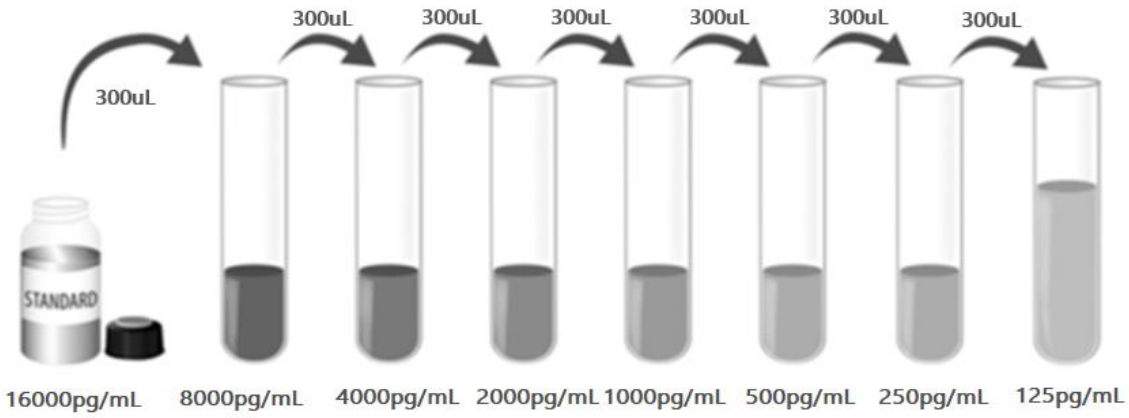
Streptavidin-HRP B (1×) - Centrifuge briefly before opening. Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

Mouse Leptin Standard - Centrifuge briefly before opening. 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 Reagent Diluent (1×) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 8000 pg/mL standard serves as the high standard. The Reagent Diluent (1×) serves as the zero standard (0 pg/mL).



D. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the 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 TMB Substrate.

VII. ASSAY PROCEDURE

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

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL of standard 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 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 Reagent 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. Protect from 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. Protect from 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 Mouse Leptin 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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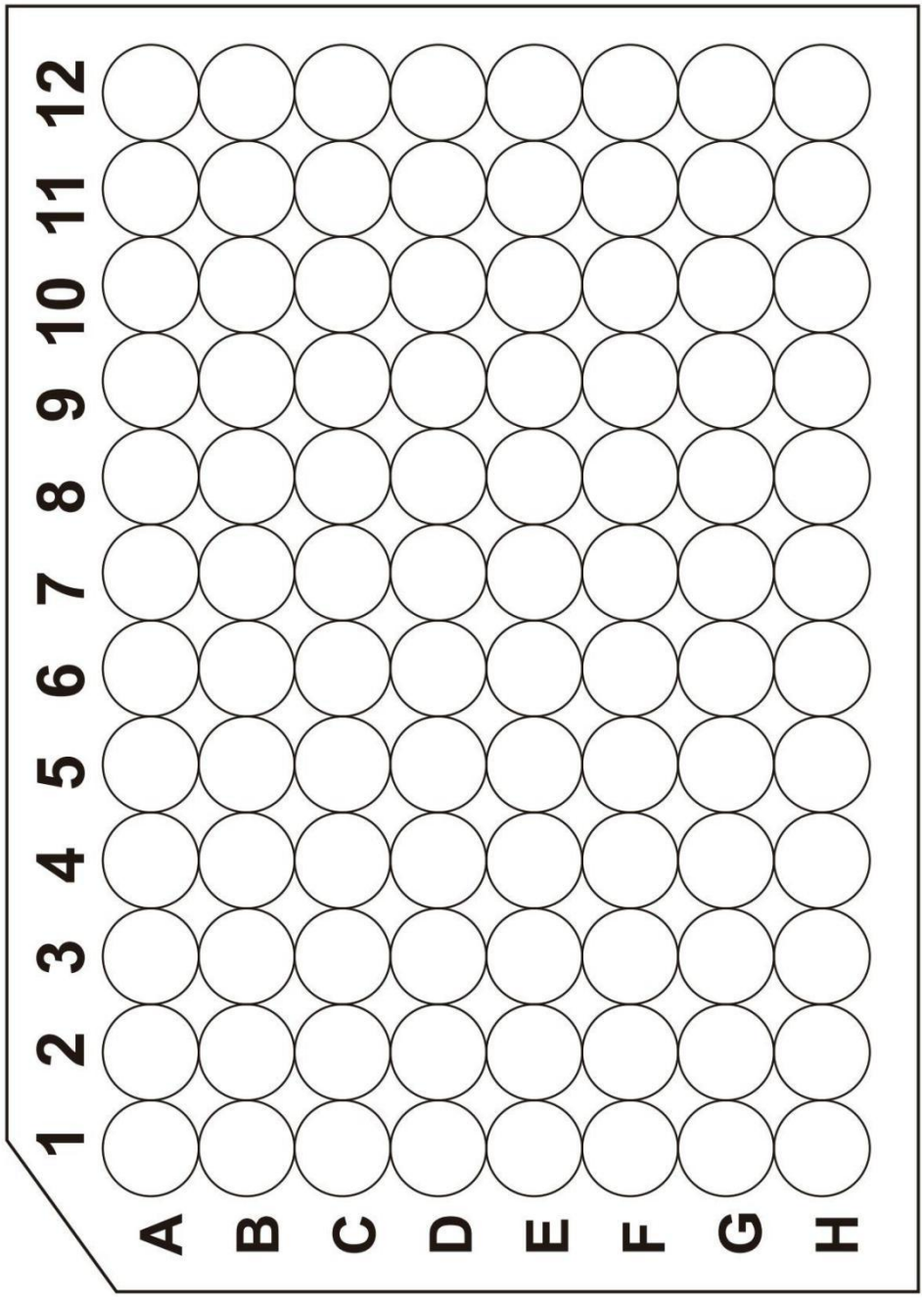
15. Houseknecht, K.L. et al. (1996) Diabetes 45:638.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 Leptin Valukine™ ELISA 试剂盒

目录号：VAL618

适用于定量检测天然和重组小鼠 Leptin 的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

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info.cn@bio-techne.com

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

小鼠瘦素是小鼠肥胖基因的蛋白质产物（1, 2）。缺乏瘦素功能的突变小鼠被发现肥胖，糖尿病，不育，减少或降低活动，代谢和体温。编码瘦素的cDNA克隆已经从人类、猿猴、小鼠和大鼠细胞中分离出来。成熟小鼠瘦素与大鼠和人类蛋白质的氨基酸序列的同源性分别为96%和84%。小鼠瘦素cDNA编码一个167个aa残基的蛋白质，包括一个信号序列为21aa的残基，和经切割后得到146个aa残基的成熟蛋白。瘦素mRNA的表达已被证明仅限于脂肪组织和胎盘中（2）。

一个高亲和力的瘦素受体（OB-R）已被克隆，它与gp130、G-CSF受体和LIF受体具有同源性（3）。OB-R的多种亚型已被鉴定，包括具有信号转导能力的大胞质结构域的长型受体（OB-R_L），和缺乏信号转导能力的短胞质结构域的受体（OB-R_S）（4-6）。还鉴定出一种缺乏跨膜结构域并可能编码受体可溶形式的OB-R转录本（7）。据报道，OB-R_L转录本主要表达在被认为对体重调节很重要的下丘脑区域。OB-R_S转录本的表达发现于多种组织中，包括脉络丛、肺癌、肾癌和原始造血细胞中（2）。OB-R已被证明是由小鼠糖尿病（db）和大鼠脂肪（fa）基因编码（8）。长期以来，已知db或fa突变的纯合啮齿类动物表现出几乎与ob/ob小鼠相同的肥胖表型（9）。

在小鼠、大鼠和人的血清或血浆中检测到高瘦素水平（ng/mL）。瘦素的循环水平被证明是受到各种刺激反应的调节，包括食物摄入、胰岛素、糖皮质激素、细胞因子和生殖事件（2, 10-14）。血清中大多数瘦素被报道可与多种瘦素结合蛋白结合（15）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠Leptin抗体包被于微孔板上，样品和标准品中的小鼠Leptin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小鼠Leptin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。洗涤未结合试剂后，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小鼠血清样本；
- 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，应将样本用试剂稀释液（1×）稀释后重新检测；
- 检测结果的不同可由多种因素引起，包括实验人员的操作、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)	180.4	1248	4429	186.2	1271.8	4464.1
标准差	4.7	33.3	113.9	8.2	54.2	130.6
CV%	2.6	2.7	2.6	4.4	4.3	2.9

B. 回收率

在不同样本中掺入检测范围内不同水平的小鼠Leptin，测定其回收率。回收率范围在85.2-105.7%，平均回收率在96.4%。

C. 灵敏度

小鼠Leptin的最低可测剂量（MDD）一般小于5.35 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*E.coli*表达的高纯度重组小鼠Leptin蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠Leptin，然后用试剂稀释液（1×）将样本稀释到检

测范围内，测定其线性。

稀释倍数	平均值/期待值 (%)	范围 (%)
1:2	100.0	93.6 - 106.1
1:4	99.1	94.6 - 104.0
1:8	94.7	89.7 - 100.8
1:16	92.5	87.5 - 97.2

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 mouse	Recombinant human
Leptin R	Leptin R

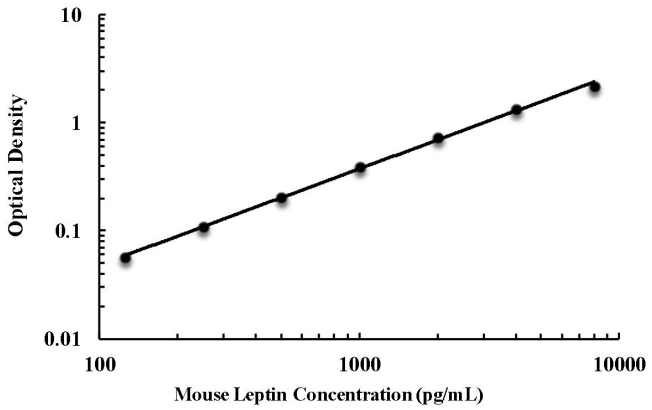
人Leptin重组蛋白浓度为50 ng/mL时检测值为94 pg/mL（0.19%交叉反应）。

大鼠Leptin重组蛋白浓度为1.6 ng/mL时检测值为651 pg/mL（42%交叉反应）。

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。



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1000	0.434 0.415	0.424	0.389
2000	0.782 0.738	0.760	0.725
4000	1.345 1.408	1.376	1.341
8000	2.194 2.233	2.213	2.178

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse Leptin Microplate	包被抗小鼠 Leptin 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Mouse Leptin Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Mouse Leptin Detection antibody	生物素化的小鼠 Leptin 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Reagent Diluent Concentrate 3 (5×)	浓缩的稀释液（5×）用于配制试剂稀释液（1×），以稀释标准品、样品、检测抗体和 HRP	1 瓶
PBS Concentrate (20×)	浓缩的 PBS（20×）用于稀释试剂稀释液（1×）	1 瓶
Streptavidin-HRP B (40×)	40×浓缩的链霉亲和素标记的 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="5">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>PBS 浓缩液（20×）</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>浓缩的试剂稀释液 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，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

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。样本可能需要用试剂稀释液（1×）稀释。

血清样本: 用血清分离管(SST)分离血清。使血样室温凝集30分钟，然后1000 × g离心15分钟。吸取血清样本之后即刻用于检测，或者分装，≤-20℃贮存备用。避免反复冻融。样本可能需要用试剂稀释液（1×）稀释。

B. 样本准备工作

小鼠血清样本建议用试剂稀释液（1×）10倍稀释后进行检测，例如：20 µL血清样本+180 µL试剂稀释液（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

洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀， 待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液用蒸馏水或去离子水稀释配制成 500 mL工作浓度的洗涤液。

PBS（1×）：使用蒸馏水或去离子水稀释配制成PBS（1×）。例如，3 mL PBS浓缩液 (20×) 加入57 mL蒸馏水或去离子水稀释配制60 mL PBS（1×）。

试剂稀释液（1×）：用PBS（1×）配制试剂稀释液（1×）。例如，12 mL浓缩的试剂稀释液 3 (5×) 加入48 mL PBS (1×) 配制 60 mL试剂稀释液（1×）。

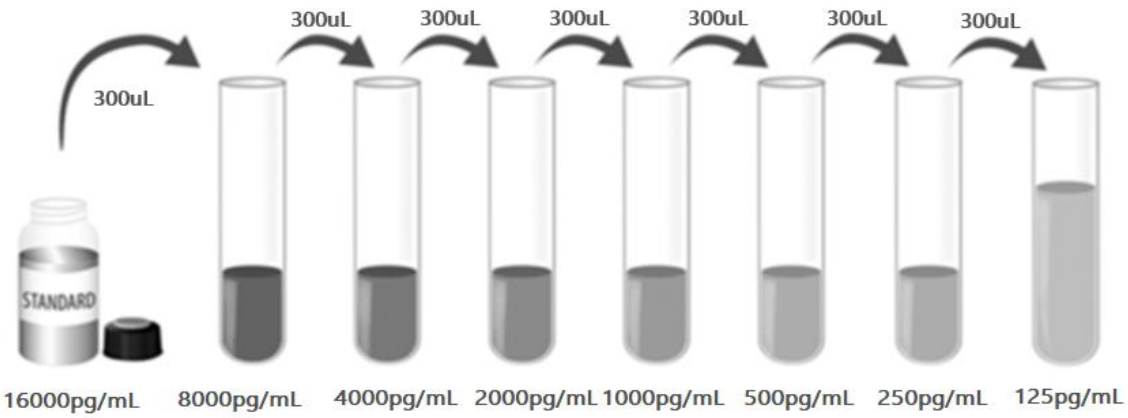
检测抗体（1×）：开盖前请瞬时离心。参考检测抗体瓶标签重溶冻干粉，制备检测抗体（100×）。轻轻震摇至少 15 分钟，其充分溶解。如有需要分装保存。用试剂稀释液（1×）稀释至检测抗体（1×），至少在使用前 15 分钟准备。

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

小鼠Leptin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16000 pg/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300 µL试剂稀释液（1×）。将标准品母液参照下图做系列稀释，每管须充分混匀后再移液到下一管。8000 pg/mL管作标准曲线最高点，试剂稀释液（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

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。数据可以通过绘制小鼠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。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。

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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