PRODUCT INFORMATION & MANUAL

Mouse Adiponectin/Acrp30 Valukine™ ELISA

Catalog Number: VAL631

For the quantitative determination of natural and recombinant mouse Adiponectin/Acrp30 concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

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Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version 202410.1

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I. BACKGROUND

Adiponectin, also known as Acrp30, apM1, AdipoQ, and GBP28, is a 30 kDa glycoprotein that is secreted primarily by adipocytes and induces wide ranging paracrine and endocrine effects on metabolism and inflammation (1-3). Mature mouse Adiponectin consists of a 66 amino acid (aa) N-terminal collagenous region and a 137 aa C-terminal C1q/TNF-α-like globular domain and shares approximately 83% and 91% amino acid (aa) sequence identity with human and rat Adiponectin, respectively (4).

Adiponectin forms 90 kDa homotrimers that contain two disulfide-linked monomers and a third subunit which is noncovalently associated. Two trimers can be covalently linked to create a 180 kDa hexamer which associates into >300 kDa high molecular weight (HMW) Adiponectin (5-7). The various forms of Adiponectin do not interconvert in the serum (7). Adiponectin is O-glycosylated on four hydroxylated lysines in its collagen domain, a modification which is required for the intracellular formation of HMW Adiponectin and its insulin-sensitizing activity (8, 9). The ratio between different forms of Adiponectin may be biologically significant; a much greater amount of HMW Adiponectin circulates in females compared to males, although the levels of trimeric and hexameric Adiponectin are comparable between genders (7, 10, 11). A cleaved form of Adiponectin, known as gAdiponectin, consists of the globular domains in trimeric complexes (12, 13). Circulating Adiponectin levels are high, comprising approximately 0.01% of total plasma protein (10). Adiponectin exerts its bioactivity through interactions with the 7-transmembrane receptors AdipoR1 and AdipoR2 (14-16). The widely expressed AdipoR1 binds gAdiponectin with high affinity but binds full length Adiponectin with very low affinity (14). AdipoR2 binds both the full length and globular forms with intermediate affinity and is relatively restricted to the liver (14). The various forms of Adiponectin also differentially interact with Cadherin-13 in muscle and with several growth factors (17, 18).

Adiponectin promotes insulin sensitivity through multiple actions on glucose and fatty acid metabolism, frequently in opposition to the actions of TNF-α (19-23). It induces a decrease in serum glucose and triglyceride levels, an increase in serum glucagon, but no change in insulin levels (20, 22, 24). In the liver, Adiponectin enhances the insulin-dependent inhibition of gluconeogenesis (22, 24). In skeletal muscle, Adiponectin promotes fatty acid uptake and oxidation, glucose uptake, and lactate

production (12, 19, 20, 25, 26). HMW Adiponectin is the most potent isoform at inducing insulin sensitization in liver, and gAdiponectin is more potent than the full length molecule at inducing metabolic effects in muscle (8, 9, 12, 25-27). The various isoforms of Adiponectin differentially trigger the activation of AMPK and NFκB in liver and muscle (6, 8, 25, 26, 28). In the adult (but not in the fetus), elevated levels of circulating total Adiponectin, and particularly HMW Adiponectin, are negatively correlated with conditions related to metabolic syndrome (10, 29). Decreased plasma HMW Adiponectin levels are associated with upper body obesity, insulin resistance, reduced fatty acid oxidation, dyslipidemia, coronary artery disease, and atherogenesis (30-33). Plasma HMW Adiponectin levels increase in response to treatment with insulin-sensitizing thiazolidinediones (27, 34).

Adiponectin inhibits inflammation by antagonizing TNF-α induced vascular endothelial cell apoptosis and the upregulation of leukocyte adhesion proteins on the vascular endothelium (32, 35, 36). In macrophages, Adiponectin promotes polarization toward the M2 anti-inflammatory phenotype, inhibits TNF-α production, and interacts with C1qRp to promote the clearance of Adiponectin-opsonized apoptotic cell debris (37-39). It protects against atherosclerosis by suppressing nitric oxide formation, the progression of macrophages into foam cells, and the migration of adventitial fibroblasts to the intima (40, 41). In nonmetabolic disorders such as rheumatoid arthritis and inflammatory bowel disease, however, Adiponectin levels are elevated and it can promote inflammation (42-45). Adiponectin also negatively regulates myelomonocytic progenitor cell growth (38).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse Adiponectin has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any mouse Adiponectin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse Adiponectin 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 mouse Adiponectin 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 supernates, mouse plasma, mouse serum and tissue homogenates.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent (1×) and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty-three separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
Mean (ng/mL)	0.33	1.23	3.83	0.35	1.22	4.02
Standard Deviation	0.022	0.072	0.221	0.021	0.061	0.258
CV%	6.7	5.9	5.8	6.0	5.0	6.4

B. RECOVERY

The recovery of mouse Adiponectin spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range (%)
Cell culture media (n=7)	103	82-115
Mouse serum* (n=6)	98	85-114
Mouse EDTA plasma* (n=4)	92	81-106
Mouse heparin plasma* (n=4)	93	77-110

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

C. SENSITIVITY

Fourteen assays were evaluated and the minimum detectable dose (MDD) of mouse Adiponectin ranged from 0.001-0.007 ng/mL. The mean MDD was 0.003 ng/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 NS0-expressed recombinant mouse Adiponectin produced at R&D Systems. The recombinant mouse Adiponectin preparation contains a mixture of the trimeric, hexameric, and higher order multimeric full-length Adiponectin isoforms.

E. LINEARITY

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

Dilution		Cell culture media (n=4)	Tissue homogenates* (n=2)	Mouse serum* (n=6)	Mouse EDTA plasma* (n=3)	Mouse heparin plasma* (n=3)
1:2	Average % of Expected	93	97	97	95	95
	Range (%)	88-105	90-103	92-102	95-95	93-98
1:4	Average % of Expected	94	104	100	97	98
	Range (%)	88-105	98-109	94-103	93-100	97-99
1:8	Average % of Expected	95	108	101	96	96
	Range (%)	87-108	99-116	99-104	93-99	94-97
1:16	Average % of Expected	114	109	101	97	96
	Range (%)	114-114	101-117	97-107	93-101	93-100

*Samples were diluted prior to assay.

F. SAMPLE VALUES

Mouse serum/plasma - Samples were evaluated for detectable levels of mouse Adiponectin in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Mouse serum (n=20)	7476	2652-15528	2959
Mouse EDTA plasma (n=10)	6675	4408-8880	1423
Mouse heparin plasma (n=10)	7198	5228-9702	1494

Cell Culture Supernates - Two lungs (1-2 mm pieces in 40 mL of medium) were cultured for 7 days in RPMI supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed for evaluation, assayed for levels of mouse Adiponectin, and measured 1.4 ng/mL.

Tissue Homogenates - Homogenates from spleen, liver, and fat tissue were assayed for mouse Adiponectin and measured 13 ng/mL, 30 ng/mL, and 51 ng/mL, respectively.

G. SPECIFICITY

This assay recognizes natural and recombinant full-length mouse Adiponectin.

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 the same concentrations in a mid-range mouse Adiponectin control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:		**Recombinant human:**
CD27 Ligand	OX40 Ligand	Adiponectin
CD30 Ligand	RANK Ligand	C1qR/Fc Chimera
CD40 Ligand	TRAIL	
Fas Ligand	TNF-α	
LT-α1/β2	TNF-α (truncated)	
LT-α2/β1	TWEAK	

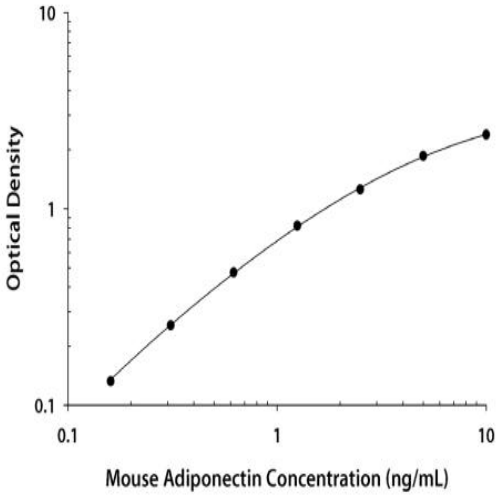
This assay does not recognize mouse gAdiponectin.

This assay does not detect recombinant rat Adiponectin (up to 2 µg/mL) or rat serum samples.

IV. EXPERIMENT

EXAMPLE STANDARD

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.010 0.011	0.010	—
0.156	0.138 0.147	0.142	0.132
0.313	0.259 0.271	0.265	0.255
0.625	0.469 0.498	0.484	0.474
1.25	0.808 0.850	0.829	0.819
2.5	1.219 1.311	1.265	1.255
5	1.856 1.878	1.867	1.857
10	2.386 2.403	2.394	2.384

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse Adiponectin Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse Adiponectin.	1 plate
Mouse Adiponectin Conjugate	An antibody specific for mouse Adiponectin conjugated to horseradish peroxidase.	1 vial
Mouse Adiponectin Standard	Recombinant mouse Adiponectin in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Mouse Adiponectin Control	Recombinant mouse Adiponectin in a buffered protein base; lyophilized. The assay value of the Control should be within the range specified on the label.	1 vial
Assay Diluent RD1W	A buffered protein base.	1 vial
Calibrator Diluent Concentrate (4×)/ RD5-26	A 4× concentrated buffered protein base used to dilute standard and samples.	2 vials
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant.	1 vial
TMB Substrate	TMB ELISA Substrate Solution/TMB Substrate Solution.	2 vial
Stop Solution	Diluted hydrochloric 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>Wash Buffer (1×)</td><td rowspan="7">May be stored for up to 1 month at 2-8°C.*</td></tr>
<tr><td>Assay Diluent RD1W</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Conjugate</td></tr>
<tr><td>TMB Substrate</td></tr>
<tr><td>Control</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Calibrator Diluent Concentrate (4×)/ RD5-26</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.
- Test tubes for dilution of standards and samples.

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×).

Tissue Homogenates - Organs from 2-5 mice were rinsed with PBS to remove excess blood, chopped into 1-2 mm pieces, homogenized in 5-10 mL of PBS in a tissue homogenizer, and stored at ≤ -20 °C overnight. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g to remove particulate. Fat tissue collected from 3 female mice was homogenized in 5-10 mL of PBS and stored at ≤ -20 °C overnight. Homogenates were centrifuged for 5 minutes at 5000 x g. Samples may require dilution with Calibrator Diluent (1×).

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 × 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 or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x 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: *Citrate plasma has not been validated for use in this assay.*

B. SAMPLE PREPARATION

Mouse serum and plasma samples recommend a 2000-fold dilution. A suggested 2000-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent (1×). Complete the 2000-fold dilution by adding 10 µL of the diluted sample + 190 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse Adiponectin Control - Reconstitute the Control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

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

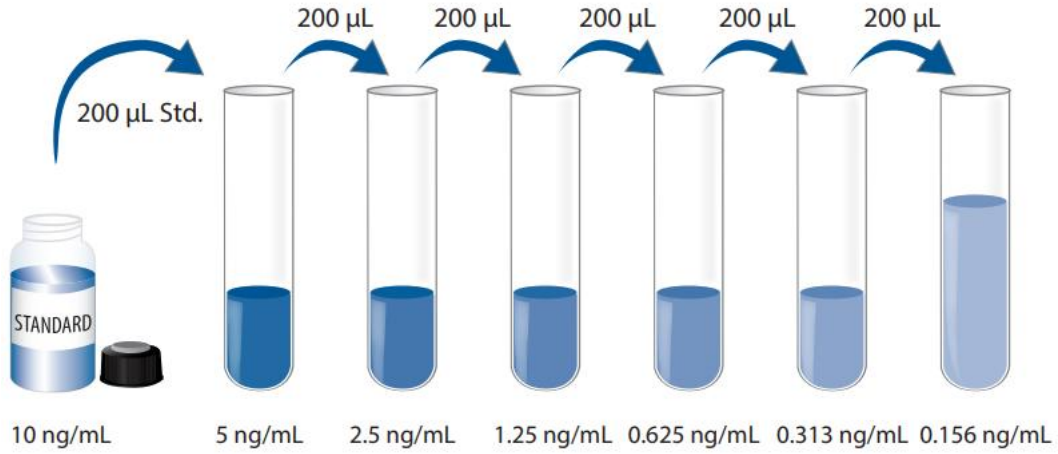
of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

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

Mouse Adiponectin Standard- Refer to the vial label for the reconstitution volume* This reconstitution produces a stock solution of 10 ng/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 200 µL of 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 undiluted standard serves as the high standard (10 ng/mL). The Calibrator Diluent (1×) serves as the zero standard (0 ng/mL).



D. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- TMB Substrate should remain colorless until added to the plate. Keep TMB Substrate protected from light. TMB Substrate should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.

VII.ASSAY PROCEDURE

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

1. Prepare reagents, standard dilutions, control, and samples 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 RD1W to each well.
4. Add 50 µL of standard, control and prepared sample per well. Tap plate gently for one minute. Cover with the adhesive strip provided. **Incubate for 3 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 four times for a total of five 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 100 µL of Mouse Adiponectin 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 100 µL of TMB Substrate to each well. **Incubate for 30 minutes at room temperature. Protect from light**.
9. Add 100 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, 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 mouse Adiponectin 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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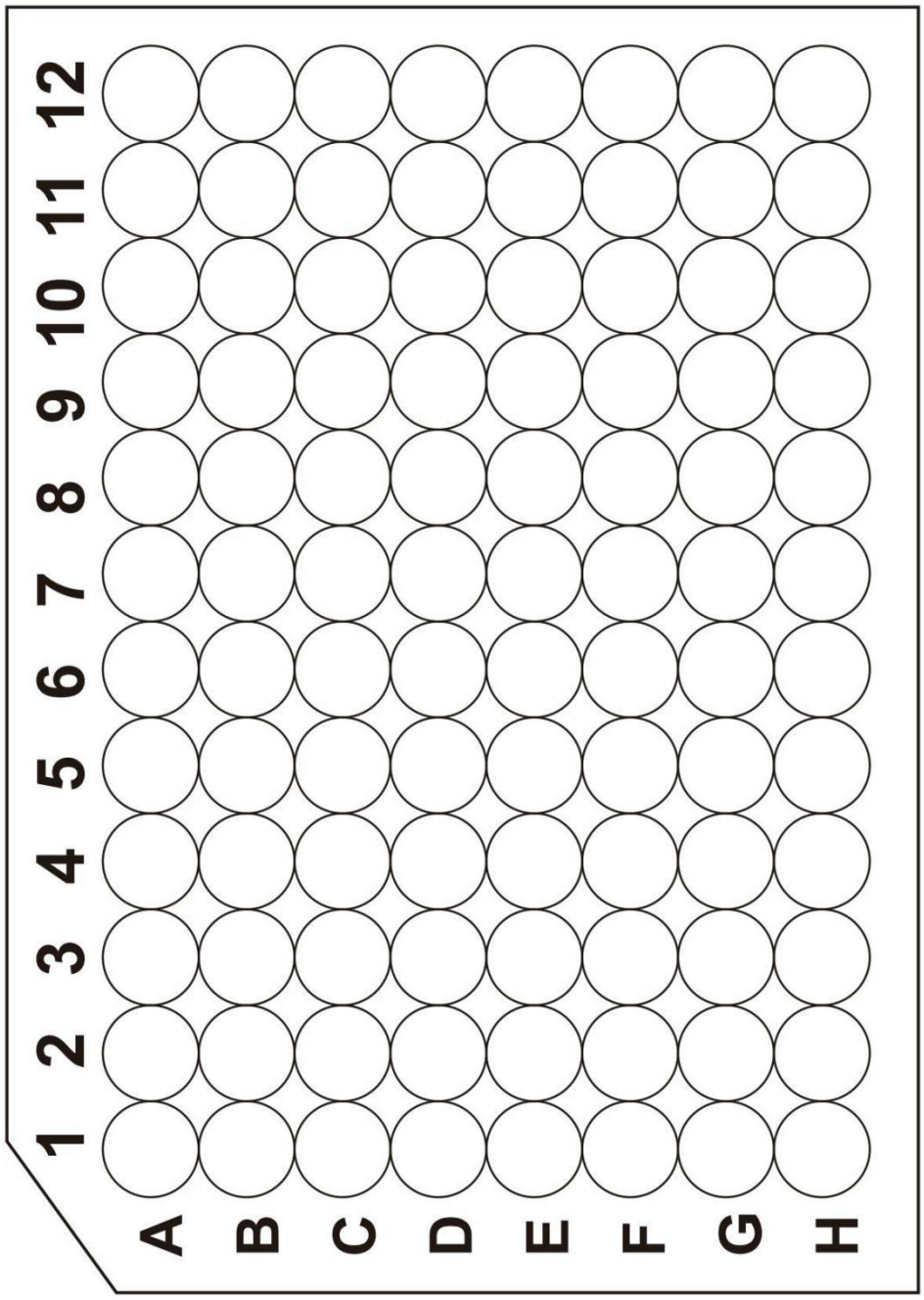
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 Adiponectin/Acrp30 Valukine™ ELISA 试剂盒

目录号：VAL631

适用于定量检测天然和重组小鼠 Adiponectin/Acrp30 的浓度

科研专用，不可用于临床诊断

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版本号 202410.1

目录

I. 背景

脂联素，也称为Acrp30，apM1，AdipoQ和GBP28，是一种30 kDa的糖蛋白，主要由脂肪细胞分泌，对新陈代谢和炎症有广泛的旁分泌和内分泌作用(1-3)。成熟的小鼠脂联素由66个氨基酸（amino acid, aa）的N端胶原区和137个aa的C端C1q/TNF-α样球状结构域组成，与人类和大鼠的脂联素分别有大约83%和91%的氨基酸序列相同性（4）。脂联素可形成90 kDa的同源三聚体，其中包含两个二硫键连接的单体和第三个非共价连接的亚基。两个三聚体可通过共价连接形成180 kDa的六聚体，并结合成大于300 kDa的高分子量（high molecular weight, HMW）脂联素（5-7）。各种形式的脂肪连通素在血清中不会相互转化（7）。脂联素在其胶原结构域的四个羟基赖氨酸上有O-糖基化，这种修饰是细胞内形成高分子量脂联素及其胰岛素敏感活性所必需的（8, 9）。不同形式的脂联素之间的比率可能具有重要的生物学意义；与男性相比，女性体内循环的HMW脂联素要多得多，尽管两性之间三聚体和六聚体脂联素的水平相当（7, 10, 11）。脂联素的裂解形式，称为gAdiponectin，由三聚体复合物中的球状结构域组成（12, 13）。循环脂联素水平很高，约占总血浆蛋白的0.01％（10）。脂联素通过与7种跨膜受体AdipoR1和AdipoR2的相互作用发挥其生物活性（14-16）。广泛表达的AdipoR1与gAdiponectin的结合亲和力很高，但与全长脂联素的结合亲和力很低（14）。AdipoR2与全长和球状脂联素结合的亲和力居中，并且相对局限于肝脏（14）。不同形式的脂联素还与肌肉中的Cadherin-13和几种生长因子发生不同的相互作用（17, 18）。

脂联素通过对葡萄糖和脂肪酸代谢的多种作用促进胰岛素敏感性，经常与TNF-α的作用相反（19-23）。它能降低血清葡萄糖和甘油三酯水平，增加血清胰高血糖素，但胰岛素水平没有变化（20, 22, 24）。在肝脏中，脂联素能增强胰岛素对葡萄糖生成的抑制作用（22、24）。在骨骼肌中，脂联素促进脂肪酸摄取和氧化、葡萄糖摄取和乳酸生成（12, 19, 20, 25, 26）。HMW脂联素是诱导肝脏胰岛素敏感化的最有效异构体，而gAdiponectin在诱导肌肉代谢效应方面比全长分子更有效（8, 9, 12, 25-27）。在肝脏和肌肉中，不同异构体的脂联素会不同程度地触发AMPK和NFκB的激活（6, 8, 25, 26, 28）。在成人（而非胎儿）中，循环中总的脂联素，尤其是HMW脂联素水平的升高与代谢综合征的相关情况呈负相关（10, 29）。血浆HMW脂联素水平的降低与上半身肥胖、胰岛素抵抗、脂肪酸氧化减少、血脂异常、冠状动脉疾病和动脉粥样硬化有关（30-33）。使用胰岛素敏感性噻唑烷二酮类药物治疗后，血浆HMW脂联素水平会升高（27, 34）。脂联素通过拮抗TNF-α诱导的血管内皮细胞凋亡和血管内皮上白细胞粘附蛋白的上调抑制炎症（32, 35, 36）。在巨噬细胞中，脂联素可促进巨噬细胞向M2抗炎表型极化，抑制TNF-α的产生，并与C1qRp相互作用，促进脂联素凋亡细胞碎片的清除（37-39）。脂联素通过抑制一氧化氮的形成、巨噬细胞向泡沫细胞的发展以及血管内壁成纤维细胞向血管内壁的迁移，从而防止动脉粥样硬化（40, 41）。然而，在类风湿性关节炎和炎症性肠病等非代谢性疾病中，脂联素水平升高，并可促进炎症（42-45）。脂联素还能负向调节髓单核细胞祖细胞的生长（38）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠Adiponectin抗体包被于微孔板上，样品，质控品和标准品中的小鼠Adiponectin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小鼠Adiponectin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，小鼠血浆样本，小鼠血清样本和组织匀浆样本；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释液（1×）稀释后重新检测；
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的效期等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测20次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板间分别检测23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 (ng/mL)	0.33	1.23	3.83	0.35	1.22	4.02
标准差	0.022	0.072	0.221	0.021	0.061	0.258
CV%	6.7	5.9	5.8	6.0	5.0	6.4

B. 回收率

在不同类型样本中掺入检测范围内不同水平的小鼠Adiponectin，测定其回收率。

样本类型	平均回收率%	范围 (%)
细胞培养基 (n=7)	103	82-115
小鼠血清样本* (n=6)	98	85-114
小鼠EDTA血浆样本* (n=4)	92	81-106
小鼠肝素血浆样本* (n=4)	93	77-110

*样品在检测前按照样品制备部分的指示进行样本稀释

C. 灵敏度

14次检测结果表明，小鼠Adiponectin的最低可测剂量（MDD）范围为0.001- 0.007 ng/mL。平均MDD为0.003 ng/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NS0表达的重组小鼠Adiponectin校正。重组小鼠Adiponectin含有三聚体、六聚体和更高阶多聚体全长Adiponectin亚型的混合物。

E. 线性

不同的样本中含有或掺入高浓度的小鼠Adiponectin，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=4)	组织匀浆* (n=2)	小鼠血清* (n=6)	小鼠EDTA血浆* (n=3)	小鼠肝素血浆* (n=3)
1:2	平均值/期待值（%）	93	97	97	95	95
	范围（%）	88-105	90-103	92-102	95-95	93-98
1:4	平均值/期待值（%）	94	104	100	97	98
	范围（%）	88-105	98-109	94-103	93-100	97-99
1:8	平均值/期待值（%）	95	108	101	96	96
	范围（%）	87-108	99-116	99-104	93-99	94-97
1:16	平均值/期待值（%）	114	109	101	97	96
	范围（%）	114-114	101-117	97-107	93-101	93-100

*样本在测定前被稀释

F. 样本预值

小鼠血清/血浆样本 - 在此测定中对样品进行小鼠Adiponectin的可检测水平的评估。

样本类型	平均值 (ng/mL)	范围 (ng/mL)	标准差(ng/mL)
小鼠血清样本 (n=20)	7476	2652-15528	2959
小鼠EDTA血浆样本 (n=10)	6675	4408-8880	1423
小鼠肝素血浆样本 (n=10)	7198	5228-9702	1494

细胞上清样本 - 将2个肺组织（1 - 2 mm的小块）培养在含10%胎牛血清的40mL RPMI培养基中，培养7天。取等量的细胞培养上清，检测小鼠Adiponectin的含量，检测值为1.4 ng/mL。

组织匀浆样本 - 脾、肝和脂肪组织匀浆中小鼠Adiponectin的含量分别为13 ng/mL、30 ng/mL和51 ng/mL。

G. 特异性

此ELISA法可检测天然及重组全长小鼠Adiponectin。

将以下因子用标准品稀释液（1×）配制成50 ng/mL的浓度来检测与小鼠Adiponectin的交叉反应。将50 ng/mL的干扰因子掺入中间范围的重组小鼠Adiponectin对照品中，来检测对小鼠Adiponectin的干扰。没有观察到明显的交叉反应或干扰。

Recombinant mouse:		**Recombinant human:**
CD27 Ligand	OX40 Ligand	Adiponectin
CD30 Ligand	RANK Ligand	C1qR/Fc Chimera
CD40 Ligand	TRAIL	
Fas Ligand	TNF-α	
LT-α1/β2	TNF-α(truncated)	
LT-α2/β1	TWEAK	

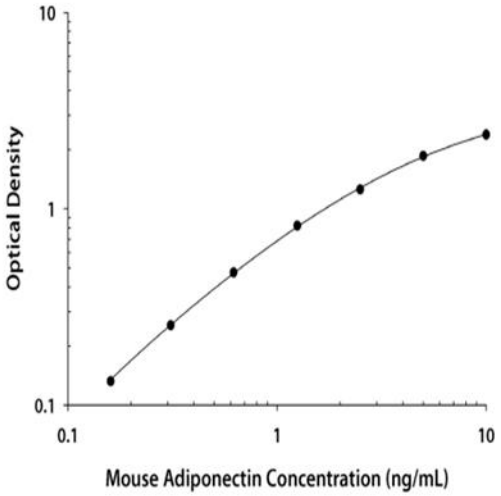
本试剂盒不能识别小鼠gAdiponectin。

本试剂盒不能检测大鼠Adiponectin（高达2 μg/mL）或大鼠血清样本。

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。



(ng/mL)	O.D.	Average	Corrected
0	0.010 0.011	0.010	—
0.156	0.138 0.147	0.142	0.132
0.313	0.259 0.271	0.265	0.255
0.625	0.469 0.498	0.484	0.474
1.25	0.808 0.850	0.829	0.819
2.5	1.219 1.311	1.265	1.255
5	1.856 1.878	1.867	1.857
10	2.386 2.403	2.394	2.384

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse Adiponectin Microplate	包被抗小鼠Adiponectin抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Mouse Adiponectin Conjugate	酶标检测抗小鼠Adiponectin抗体	1瓶
Mouse Adiponectin Standard	小鼠Adiponectin标准品（冻干），参考瓶身标签进行重溶	1瓶
Mouse Adiponectin Control	小鼠Adiponectin质控品（冻干），质控品的测定值应在标签上规定的范围内	1瓶
Assay Diluent RD1W	检测液	1瓶
Calibrator Diluent Concentrate (4×)/ RD5-26	浓缩标准品稀释液（4×）用于稀释标准品和样本	2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℃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="6">2-8℃储存，最多30天*</td></tr>
<tr><td>检测液RD1W</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>质控品</td></tr>
<tr><td>标准品</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>浓缩标准品稀释液（4×）/RD5-26</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
- 100mL和500mL量筒
- 用于稀释标准品和样品的管子

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：颗粒物应通过离心去除；立即检测样本或分装，≤ -20 °C储存备用，，避免反复冻融。样本可能需要用标准品稀释液（1×）稀释。

组织匀浆：用PBS冲洗2-5只小鼠的器官，以去除多余的血液，切成1-2 mm的碎片，在组织匀浆器中用5-10 mL PBS匀浆，并在≤ -20℃下储存过夜。在进行两次冻融循环以打破细胞膜后，将匀浆在5000 × g下离心5分钟以去除颗粒。将以3只雌性小鼠收集的脂肪组织匀浆于5-10 mL PBS中，并在≤ -20℃下储存过夜。匀浆在5000 × g下离心5分钟。样品可能需要用标准品稀释液（1×）稀释。

血清样本：血液样品在室温下凝集2小时，然后在2000 × g下离心20分钟。吸取血清样本之后即刻用于检测，或者分装，≤ -20℃储存备用。避免反复冻融。样本可能需要用标准品稀释液（1×） 稀释。

血浆样本：使用EDTA或肝素作为抗凝剂收集血浆。然后2000 × g离心20分钟。需在30分钟内收集血浆样本之后即刻用于检测，或者分装，≤ -20℃储存备用。避免反复冻融。样本可能需要用稀释液（1×）稀释。

注意：*本试剂盒对柠檬酸钠血浆尚未被验证。*

B. 样本准备工作

小鼠血清样本和血浆样本建议用标准品稀释液（1×）2000倍稀释后进行检测，即10 µL血清+990 µL标准品稀释液（1×）。然后10 µL稀释后样品+190µL标准品稀释液（1×），即是2000倍稀释。 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。

小鼠Adiponectin质控品：使用1.0 mL去离子水或蒸馏水重溶质控品。测定时不稀释质控品。

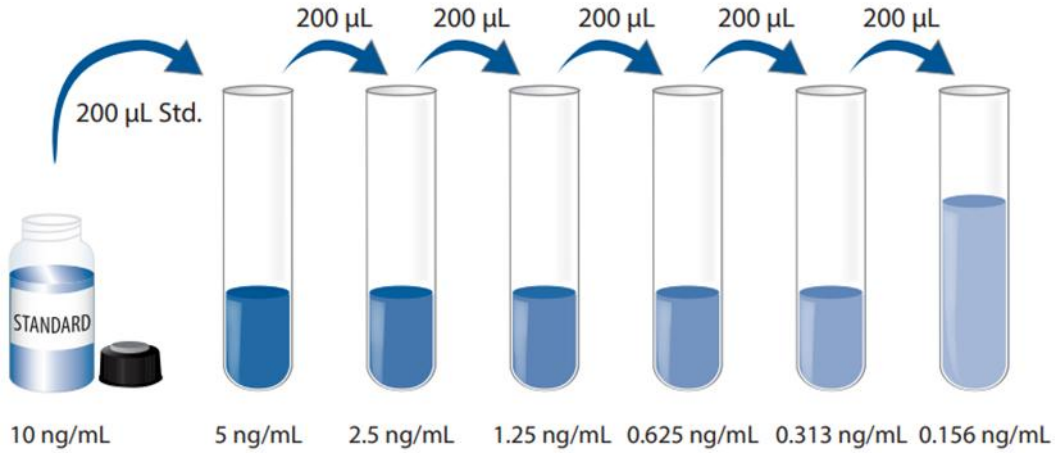
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

标准品稀释液（1×）：使用蒸馏水或去离子水稀释配制成标准品稀释液（1×）。

小鼠Adiponectin标准品：重溶体积请参考瓶身标签*，得到浓度为10 ng/mL标准品母液。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。

每个稀释管中加入200 µL标准品稀释液（1×）。将标准品母液参照下图做系列稀释，每管须充分混匀后再移液到下一管。没有稀释的标准品母液可用作标准曲线最高点（10 ng/mL），标准品稀释液（1×）可用作标准曲线零点（0 ng/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，标准品和质控品做复孔检测。

1. 按照上一节的说明，准备好所有需要的试剂，标准品，质控品和样本；
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. 每孔加入50μL检测液RD1W。
4. 分别将不同浓度标准品，质控品和实验样本加入相应孔中，每孔50 μL。轻轻拍微孔板1分钟，后用封板膜封住反应孔，**室温孵育3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4次，共洗5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100 μL小鼠Adiponectin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. 在每个微孔内加入100 μL TMB底物溶液，**室温孵育30分钟。注意避光；**
9. 在每个微孔内加入100 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，质控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Adiponectin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

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