PRODUCT INFORMATION & MANUAL

Mouse Lipocalin-2/NGAL Valukine™ ELISA

Catalog Number: VAL634

For the quantitative determination of natural and recombinant mouse Lipocalin-2/NGAL 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

Members of the Lipocalin family have limited sequence identity but share a highly conserved fold with an eight-stranded anti-parallel β barrel motif that encloses an internal ligand-binding site. They are known for their actions as transporters that carry small hydrophobic molecules such as steroid hormones, vitamins, odorants, and metabolic products (1). Lipocalin-2, also known as Neutrophil Gelatinase-associated Lipocalin (NGAL), Siderocalin, and 24p3, was originally identified as a component of neutrophil granules (2). Its synthesis is induced in macrophages, glial cells, and epithelial cells during inflammation (3-5). Lipocalin-2 has been implicated in a variety of cellular processes including the innate immune response, differentiation, tumorigenesis, and cell survival. It is a 25 kDa glycoprotein that exists in monomeric and homodimeric forms and associates covalently with MMP-9 (2, 6). Its association with MMP-9 may modulate protease activity by protecting MMP-9 from degradation (6, 7). Mature mouse Lipocalin-2 shares 62% and 81% amino acid sequence identity with human and rat Lipocalin-2, respectively.

Lipocalin-2 binds catecholate siderophores which are secreted by bacteria and required for bacterial iron uptake. Lipocalin-2 functions as a bacteriostatic agent in the innate immune response by limiting the bacterial iron supply (8, 9). TLR4 activation induces its production in immune and epithelial cells, and Lipocalin-2 knockout mice are impaired in resisting bacterial infection (3, 9-11). Some virulent bacterial strains evade immune clearance by producing modified siderophores that are not bound by Lipocalin-2 (12, 13). Iron uptake by Lipocalin-2 into mammalian cells is important for the regulation of iron-sensitive gene transcription (14). In the kidney, Lipocalin-2-mediated iron trafficking is required for protection from renal injury (15). Megalin, a member of the LDL receptor family, and 24p3 R/NGALR/BOCT have been reported as endocytic receptors for Lipocalin-2 (16, 17). In apparent contradiction, Lipocalin-2 has been shown to act as both a survival factor and a pro-apoptotic factor (5, 17-20). Its induction by pro-inflammatory cytokines may vary between mouse and human (21).

Lipocalin-2 is also upregulated in non-bacterial inflammatory pathologies such as psoriasis, ulcerative colitis, and adipose tissue in obesity where it promotes insulin resistance in hepatocytes (22-25). It is upregulated under conditions of anemia or hypoxia and inhibits the differentiation of erythrocytes (26, 27). Lipocalin-2 levels in

the urine and serum are elevated following acute renal injury (28). Lipocalin-2 is upregulated in several cancers or tumorassociated stroma, but its association with cancer is complex and may depend on tumor type or the local microenvironment (29). Lipocalin-2 has been shown to promote tumor angiogenesis, epithelial-mesenchymal transition, and metastasis in some cases (30-33) but suppress them in others (34-36).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse Lipocalin-2 has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any mouse Lipocalin-2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse Lipocalin-2 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 Lipocalin-2 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 platelet-poor plasma, mouse serum and mouse urine.
- 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)	196	476	1534	211	477	1564
Standard Deviation	13.2	19.6	65.9	15.2	28.6	98.5
CV%	6.7	4.1	4.3	7.2	6.0	6.3

B. RECOVERY

The recovery of mouse Lipocalin-2 spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range (%)
Cell culture media (n=4)	93	82-101

C. SENSITIVITY

Fifty-four assays were evaluated and the minimum detectable dose (MDD) of mouse Lipocalin-2 ranged from 1.40 to 8.80 pg/mL . The mean MDD was 3.18 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 NS0-derived recombinant mouse Lipocalin-2 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 Lipocalin-2 and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=4)	Mouse serum* (n=4)	Platelet-poor		Mouse urine* (n=4)
				Mouse EDTA plasma* (n=4)	Mouse heparin plasma* (n=4)	
1:2	Average % of Expected	100	101	106	103	103
	Range (%)	96-103	99-103	100-110	98-108	93-116
1:4	Average % of Expected	103	106	104	101	102
	Range (%)	98-106	101-115	96-114	91-109	93-112
1:8	Average % of Expected	108	105	105	101	102
	Range (%)	100-112	99-114	91-117	90-110	95-111
1:16	Average % of Expected	106	101	104	98	105
	Range (%)	94-118	95-118	90-118	91-108	96-111

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

F. SAMPLE VALUES

Mouse serum/Platelet-poor plasma/Urine - Samples were evaluated for detectable levels of mouse Lipocalin-2 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Mouse serum (n=20)	141	58.3-290	60.2
Mouse platelet-poor EDTA plasma (n=20)	76.0	40.7-143	30.1
Mouse platelet-poor heparin plasma (n=20)	54.1	32.5-74.7	12.3
Mouse urine (n=20)	48.6	12.9-168	34.1

Cell Culture Supernates:

J774A.1 mouse reticulum cell sarcoma macrophage cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL of penicillin, and 100 µg/mL of streptomycin sulfate. Cells were either untreated or stimulated with 100 ng/mL of recombinant mouse IFN-γ and 1 µg/mL of lipopolysaccharide (LPS) for 2 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse Lipocalin-2.

Condition	Observed Levels (ng/mL)
Unstimulated	0.817
Stimulated	161

WEHI-3 myelomonocytic leukemia cells (2 x 10^5 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL of penicillin, and 100 µg/mL of streptomycin sulfate. Cells were either untreated or stimulated with 1 µg/mL of LPS for 4 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse Lipocalin-2.

Condition	Observed Levels (ng/mL)
Unstimulated	83.7
Stimulated	178

Two mouse brains and kidneys were homogenized and seeded in RPMI 1640 supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM

L-glutamine, 100 U/mL of penicillin, and 100 µg/mL of streptomycin sulfate. The resulting supernates were either untreated or stimulated with 1 µg/mL of LPS for 3 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse Lipocalin-2.

Condition	Observed Levels (ng/mL)
Brain unstimulated	0.334
Brain stimulated	2.01
Kidney unstimulated	1.28
Kidney stimulated	4.69

G. SPECIFICITY

This assay recognizes natural and recombinant mouse Lipocalin-2.

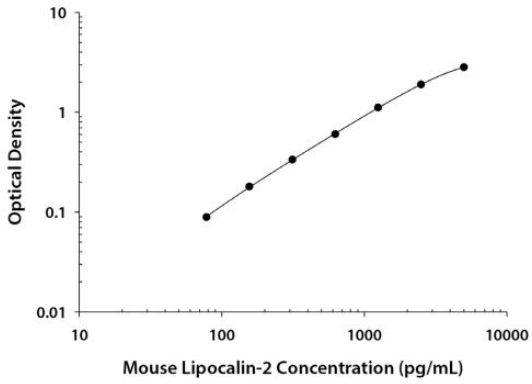
The factors listed below were prepared at 200 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following factors at 200 ng/mL in a mid-range mouse Lipocalin-2 Standard were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:	Recombinant rat:
MMP-2	Lipocalin-2/NGAL
MMP-3	MMP-9
MMP-7	**Recombinant human:**
MMP-8	Lipocalin-1
MMP-9	Lipocalin-2/NGAL
MMP-12	MMP-9

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	0.014 0.016	0.015	—
78.1	0.099 0.109	0.104	0.089
156	0.193 0.197	0.195	0.180
313	0.340 0.360	0.350	0.335
625	0.610 0.625	0.618	0.603
1250	1.120 1.135	1.128	1.113
2500	1.897 1.922	1.910	1.895
5000	2.810 2.870	2.840	2.825

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse Lipocalin-2 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse Lipocalin-2.	1 plate
Mouse Lipocalin-2 Conjugate	An antibody specific for mouse Lipocalin-2 conjugated to horseradish peroxidase.	1 vial
Mouse Lipocalin-2 Standard	Recombinant mouse Lipocalin-2 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Mouse Lipocalin-2 Control	Recombinant mouse Lipocalin-2 in a buffered protein base; lyophilized. The assay value of the control should be within the range specified on the vial label.	1 vial
Assay Diluent RD1-34	A buffered protein base.	1 vial
Calibrator Diluent Concentrate (5×)/ RD5-24	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 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="9">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 RD1-34</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 (5×)/ RD5-24</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×).

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1×).

Platelet-poor Plasma - To measure circulating Lipocalin-2 levels, platelet-poor plasma is recommended. Collect plasma on ice using EDTA or heparin as an anticoagulant. Centrifuge at 2-8 °C for 15 minutes at 1000 × g within 30 minutes of collection. For complete platelet removal, an additional centrifugation step is recommended. Centrifuge the separated plasma at 2-8 °C for 10 minutes at 10,000 × g. 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*.

Urine - Collect urine using a metabolic cage. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage. Samples may require dilution with Calibrator Diluent (1×).

B. SAMPLE PREPARATION

Mouse serum and platelet-poor plasma samples recommend a 100-fold dilution. A suggested 200-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent (1×). Complete the 100-fold dilution by adding 20 µL of the diluted sample + 180 µL of Calibrator Diluent. Optimal dilutions should be determined by the end user.

Mouse urine samples recommend a 20-fold dilution. A suggested 20-fold dilution is 10 µL of 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 Lipocalin-2 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

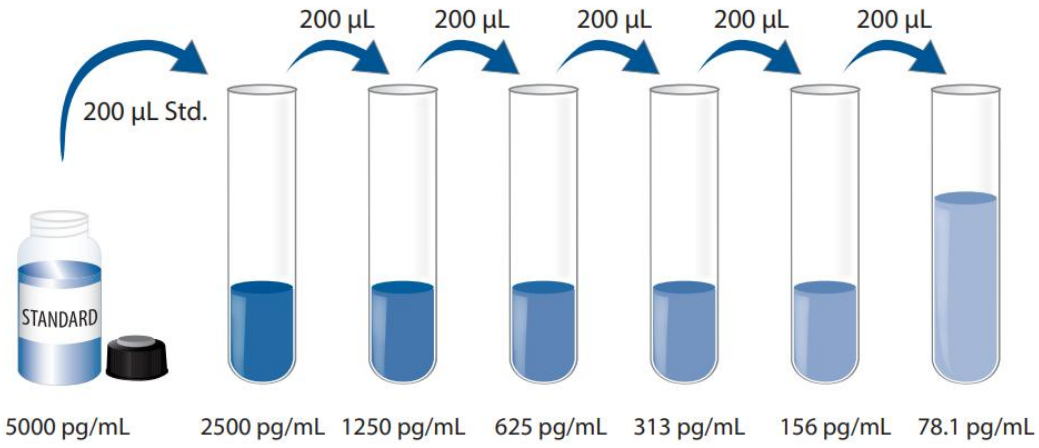
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

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

Mouse Lipocalin-2 Standard- Refer to the vial label for the reconstitution volume* Reconstitute the Mouse Lipocalin-2 Standard with Calibrator Diluent (1×). This reconstitution produces a stock solution of 5000 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 200 µL of Calibrator Diluent (1×) into each tube. Use the standard stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse Lipocalin-2 Standard (5000 pg/mL) serves as the high standard. The Calibrator Diluent (1×) serves as the zero standard (0 pg/mL).



D. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each

standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- 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, control and standards be assayed in duplicate.

1. Prepare all 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 RD1-34 to each well.
4. Add 50 µL of standard, control and prepared sample per well. Cover with the adhesive strip provided. **Incubate for 2 hours at room temperature on the benchtop.** 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 100 µL of Mouse Lipocalin-2 Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at room temperature on the benchtop**.
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 Lipocalin-2 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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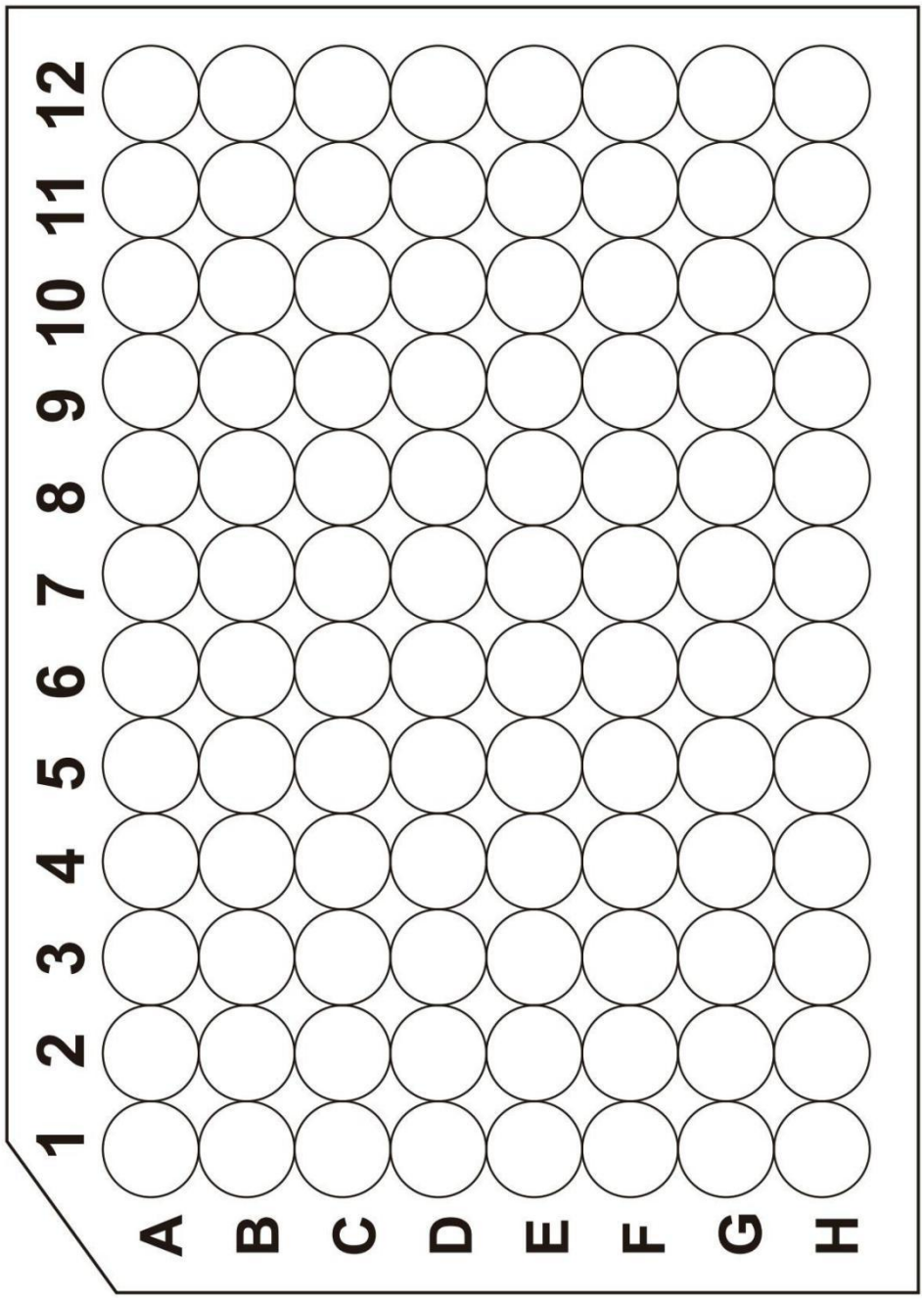
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠 Lipocalin-2/NGAL Valukine™ ELISA 试剂盒

目录号：VAL634

适用于定量检测天然和重组小鼠 Lipocalin-2/NGAL 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

脂质运载蛋白家族成员的序列相同性有限，但具有高度保守的折叠结构，即八股反平行的β桶状结构，其中包含一个内部配体结合位点。众所周知，它们是携带疏水性小分子（如类固醇激素、维生素、气味剂和代谢产物）的转运体（1）。Lipocalin-2 又称中性粒细胞明胶酶相关脂联素（NGAL）、嗜酸性粒细胞蛋白（Siderocalin）和 24p3，最初被鉴定为中性粒细胞颗粒的一种成分（2）。在炎症过程中，巨噬细胞、神经胶质细胞和上皮细胞会诱导它的合成 (3, 5)。Lipocalin-2 与多种细胞过程有关，包括先天性免疫反应、分化、肿瘤发生和细胞存活。它是一种 25 kDa 糖蛋白，以单体和同体形式存在，并与 MMP-9 共价结合（2, 6）。它与 MMP-9 的结合可能会通过保护 MMP-9 免受降解而调节蛋白酶的活性（6, 7）。成熟的小鼠 Lipocalin-2 与人和大鼠 Lipocalin-2 分别有 62% 和 81% 的氨基酸序列相同。

Lipocalin-2 结合了由细菌分泌的和细菌铁吸收所必需的儿茶素铁载体。Lipocalin-2 通过限制细菌的铁供应，在先天性免疫反应中发挥抑菌作用（8, 9）。TLR4 激活可诱导免疫细胞和上皮细胞产生 Lipocalin-2，Lipocalin-2 基因敲除小鼠抵抗细菌感染的能力受损（3, 9-11）。一些有毒的细菌菌株通过产生不受 Lipocalin-2 结合的改良的铁载体来逃避免疫清除（12, 13）。Lipocalin-2 对哺乳动物细胞的铁吸收对于调节铁敏感基因转录很重要（14）。在肾脏中，脂肪素 2 介导的铁运输需要防止肾脏损伤（15）。据报道，LDL 受体家族成员 Megalin 和 24p3 R/NGALR/BOCT 是 Lipocalin-2 的内细胞受体（16, 17）。与此明显矛盾的是，Lipocalin-2 既是一种存活因子，也是一种促凋亡因子（5, 17-20）。促炎细胞因子对它的诱导作用可能因小鼠和人类而异（21）。

Lipocalin-2 在非细菌性炎症病变中也会上调，如银屑病、溃疡性结肠炎和肥胖症中的脂肪组织，在这些病变中，Lipocalin-2 会促进肝细胞的胰岛素抵抗（22-25）。它在贫血或缺氧条件下上调，并抑制红细胞的分化（26, 27）。急性肾损伤后，尿液和血清中的 Lipocalin-2 水平会升高（28）。Lipocalin-2 在多种癌症或肿瘤相关基质中上调，但其与癌症的关系很复杂，可能取决于肿瘤类型或局部微环境（29）。Lipocalin-2 在某些情况下会促进肿瘤血管生成、上皮-间质转化和转移（30-33），但在另一些情况下则会抑制它们（34-36）。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠Lipocalin-2抗体包被于微孔板上，样品，质控品和标准品中的小鼠Lipocalin-2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Lipocalin-2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，小鼠血清样本，小鼠去血小板血浆样本和小鼠尿液样本；
- 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)	196	476	1534	211	477	1564
标准差	13.2	19.6	65.9	15.2	28.6	98.5
CV%	6.7	4.1	4.3	7.2	6.0	6.3

B. 回收率

在样本中掺入检测范围内不同水平的小鼠Lipocalin-2，测定其回收率。

样本类型	平均回收率%	范围 (%)
细胞培养基 (n=4)	93	82-101

C. 灵敏度

54次检测结果表明，小鼠Lipocalin-2的最低可测剂量（MDD）范围为1.40- 8.80 pg/mL。平均MDD为3.18 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NS0衍生的重组小鼠Lipocalin-2校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠Lipocalin-2，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)	小鼠血清* (n=4)	去血小板		小鼠尿液* (n=4)
				小鼠EDTA血浆* (n=4)	小鼠肝素血浆* (n=4)	
1:2	平均值/期待值（%）	100	101	106	103	103
	范围（%）	96-103	99-103	100-110	98-108	93-116
1:4	平均值/期待值（%）	103	106	104	101	102
	范围（%）	98-106	101-115	96-114	91-109	93-112
1:8	平均值/期待值（%）	108	105	105	101	102
	范围（%）	100-112	99-114	91-117	90-110	95-111
1:16	平均值/期待值（%）	106	101	104	98	105
	范围（%）	94-118	95-118	90-118	91-108	96-111

*样品在检测前按照样品制备部分的指示进行样本稀释

F. 样本预值

小鼠血清/去血小板血浆样本/尿液 - 在此测定中对样品进行小鼠Lipocalin-2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)	141	58.3-290	60.2
小鼠去血小板EDTA血浆样本 (n=20)	76.0	40.7-143	30.1
小鼠去血小板肝素血浆样本 (n=20)	54.1	32.5-74.7	12.3
小鼠尿液样本 (n=20)	48.6	12.9-168	34.1

细胞上清样本：

J774A.1小鼠网细胞肉瘤巨噬细胞（1 × 10^6个/mL），培养在含有10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL硫酸链霉素的RPMI培养基中。用100 ng/mL重组小鼠IFN-γ和1 µg/mL脂多糖（LPS）刺激细胞2天或不刺激细胞。取出等量的细胞培养上清，检测小鼠Lipocalin-2的水平。

条件	**检测值 (ng/mL)**
不刺激	0.817
刺激	161

WEHI-3-单核细胞白血病细胞（2 × 10^5个/mL），培养在含有10%胎牛血清、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL硫酸链霉素的RPMI培养基中。用1 µg/mL脂多糖（LPS）刺激细胞4天或不刺激细胞。取出等量的细胞培养上清，检测小鼠Lipocalin-2的水平。

条件	**检测值 (ng/mL)**
不刺激	83.7
刺激	178

将2只小鼠的大脑和肾脏均质化，培养在含有10%胎牛血清、5 µM β-巯基乙醇、2 mM L-谷氨酰胺、100 U/mL青霉素和100 µg/mL硫酸链霉素的RPMI培养基中。得到的上清液分别以1 µg/mL LPS刺激3天或不刺激。取出等量的细胞培养上清，检测小鼠Lipocalin-2的水平

条件	**检测值 (ng/mL)**
大脑 不刺激	0.334
大脑 刺激	2.01
肾脏 不刺激	1.28
肾脏 刺激	4.69

G. 特异性

此ELISA法可检测天然及重组小鼠Lipocalin-2。

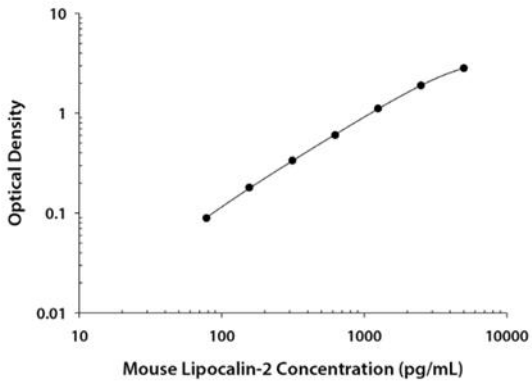
将以下因子用标准品稀释液（1×）配制成200 ng/mL的浓度来检测与小鼠Lipocalin-2的交叉反应。将200 ng/mL的干扰因子掺入中间范围的重组小鼠Lipocalin-2标准品中，来检测对小鼠Lipocalin-2的干扰。没有观察到明显的交叉反应或干扰。

Recombinant mouse:	**Recombinant rat:**
MMP-2	Lipocalin-2/NGAL
MMP-3	MMP-9
MMP-7	**Recombinant human:**
MMP-8	Lipocalin-1
MMP-9	Lipocalin-2/NGAL
MMP-12	MMP-9

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	0.014 0.016	0.015	—
78.1	0.099 0.109	0.104	0.089
156	0.193 0.197	0.195	0.180
313	0.340 0.360	0.350	0.335
625	0.610 0.625	0.618	0.603
1250	1.120 1.135	1.128	1.113
2500	1.897 1.922	1.910	1.895
5000	2.810 2.870	2.840	2.825

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse Lipocalin-2 Microplate	包被抗小鼠Lipocalin-2抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Mouse Lipocalin-2 Conjugate	酶标检测抗小鼠Lipocalin-2抗体	1瓶
Mouse Lipocalin-2 Standard	小鼠Lipocalin-2标准品（冻干），参考瓶身标签进行重溶	1瓶
Mouse Lipocalin-2 Control	小鼠Lipocalin-2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 RD1-34	检测液	1瓶
Calibrator Diluent Concentrate (5×)/ RD5-24	浓缩标准品稀释液（5×）用于稀释标准品和样本	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℃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RD1-34</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 rowspan="2">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(5×)/ RD5-24</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。

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°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。

去血小板血浆样本：为了检测Lipocalin-2水平，建议使用去血小板血浆，使用EDTA或肝素作为抗凝剂在冰上收集血浆。然后在2-8°C ，1000 × g离心15分钟，需在30分钟内收集血浆样本。为了完全去除血小板，建议进行额外的离心步骤。将分离的血浆在2-8℃ 10000 × g离心10分钟之后立即测定或者分装，≤ -20°C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。*

尿液样本：使用代谢笼收集尿液。立即通过离心和分析去除任何颗粒，或将样品分装并≤ -20°C储存备用。避免反复冻融。在测定前再次离心，以去除储存后可能出现的任何其他沉淀。样本可能需要用稀释液（1×）稀释。

B. 样本准备工作

小鼠血清样本和去血小板血浆样本建议用标准品稀释液（1×）100倍稀释后进行检测，即20 µL血清+180 µL标准品稀释液（1×）。然后20 µL稀释后样品+180µL标准品稀释液（1×），即是100倍稀释。 最佳稀释度应由最终用户确定。

小鼠尿液样本建议用标准品稀释液（1×）20倍稀释后进行检测，即10 µL血清+190 µL标准品稀释液（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。

小鼠Lipocalin-2质控品：使用1.0 mL去离子水或蒸馏水重溶质控品。混合均匀，测定时不稀释质控品。

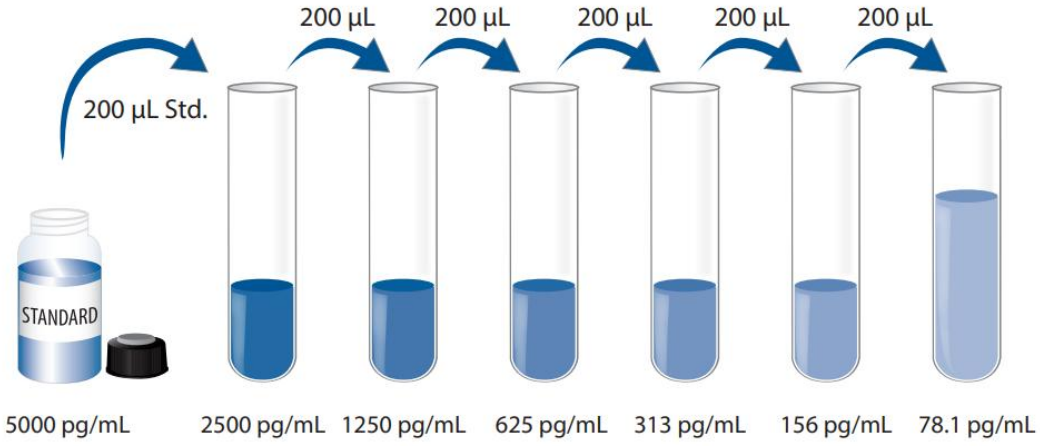
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

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Lipocalin-2标准品：重溶体积请参考瓶身标签*，用标准品稀释液（1×）重溶小鼠Lipocalin-2标准品。得到浓度为5000 pg/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（5000 pg/mL），标准品稀释液（1×）可用作标准曲线零点（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，质控品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，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RD1-34。
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。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；
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100 μL小鼠Lipocalin-2酶标检测抗体。用封板膜封住反应孔，**室温孵育2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Lipocalin-2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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