



PRODUCT INFORMATION & MANUAL

Human Lipocalin-2/NGAL Valukine™ ELISA

Catalog Number: VAL183

For the quantitative determination of natural and recombinant human 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 202309.2

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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 (1, 2). They are known for their actions as transporters that carry small hydrophobic molecules such as steroid hormones, vitamins, odorants, and metabolic products (1-4). Lipocalin-2, also known as Neutrophil Gelatinase-associated Lipocalin (NGAL) or Siderocalin, was originally identified as a component of neutrophil granules (5). Since then, its expression has been observed in most tissues, and its synthesis is induced in epithelial cells during inflammation (4, 6). Lipocalin-2 has been implicated in a variety of cellular processes including the innate immune response, differentiation, tumorigenesis, and cell survival (4, 7-9). It is a 25 kDa protein existing in monomeric, homodimeric, and heterodimeric forms, the latter in association with human matrix metalloproteinase 9 (MMP-9) (5). Its association with MMP-9 may modulate protease activity by protecting MMP-9 from degradation (10). The mouse ortholog (also known as 24p3) shares 62% sequence identity at the amino acid level (4, 5).

The functions of Lipocalin-2 continue to be elucidated. Studies indicate that it binds bacterial catecholate siderophores bound to ferric ions (9, 11). This suggests that Lipocalin-2 may act as a bacteriostatic agent by binding bacterial siderophores and limiting bacterial iron supply. This is supported by the observation that mouse Lipocalin-2 is induced in immune cells following Toll-like receptor activation, and Lipocalin-2 mouse knockouts exhibit decreased ability to counter bacterial infection (9, 12). Lipocalin-2 may also regulate iron uptake into mammalian cells (8). In the kidney, Lipocalin-2-mediated iron trafficking may be involved in both development and protection from renal injury (8, 13-15). Megalin, a member of the LDL receptor family, and 24p3 R have been reported as endocytic receptors for Lipocalin-2 (16, 17). It should be noted that the effects of Lipocalin-2 on cells might be context-dependent. For instance, it has been shown to act as both a survival factor and a pro-apoptotic factor, and its induction by pro-inflammatory cytokines may vary between mouse and human (17-19).

Lipocalin-2 has been associated with several pathological processes. For instance, it is upregulated in psoriatic skin in comparison to uninvolved control skin, and Lipocalin-2 suppresses red blood cell production in models of anemia (20, 21). Lipocalin-2 is elevated in patients with severe acute respiratory syndrome (SARS) and may act as a biomarker for acute renal injury (15, 22). It has been associated with several tumor types as well, including breast, ovarian, colorectal, and pancreatic cancers (23-26). Its function in cancer is unclear, although the invasive and metastatic behavior of tumor cells is suppressed by Lipocalin-2 in models of breast and colon cancer (27, 28).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human Lipocalin-2/NGAL has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Lipocalin-2/NGAL present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated detection antibody specific for human Lipocalin-2/NGAL is pipetted into the wells. After washing away any unbound substances, Streptavidin-HRP is pipetted into the wells. Following a wash to remove any unbound reagent, TMB substrate solution (Chromogenic agent) is added to the wells and color develops in proportion to the amount of Lipocalin-2/NGAL 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 human 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 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 separate assays to assess inter-assay precision.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
Mean (pg/mL)	614.9	152.3	37.8	616.6	150.3	37.0
Standard Deviation	31.6	7.0	1.8	29.0	7.3	2.0
CV%	5.1	4.6	4.7	4.7	4.8	5.3

B. RECOVERY

The recovery of human Lipocalin-2/NGAL spiked to different levels throughout the range of the assay in cell culture media was evaluated. The recovery ranged from 77.3 to 120.8% with an average of 98.7%.

C. SENSITIVITY

The minimum detectable dose (MDD) of human Lipocalin-2/NGAL is typically less than 2.79 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 Kit is calibrated against a highly purified NS0-expressed recombinant human Lipocalin-2/NGAL produced at R&D Systems.

E. LINEARITY

To assess the linearity of the assay, different samples were containing or spiked with high concentrations of human Lipocalin-2/NGAL and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution	Average % of Expected	Range (%)
1:2	99.9	84.2-114.7
1:4	102.7	78.9-119.9
1:8	104.8	84.2-123.7
1:16	96.6	74.5-121.0

F. SAMPLE VALUES

Cell Culture Supernates - OCI-AML5 (1×10^6 cells/mL) were cultured in α -MEM supplemented with 20% fetal bovine serum, 10 ng/mL recombinant human GM-CSF, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. Cells were cultured for 48h. Aliquots of the culture supernates were removed, assayed for levels of human Lipocalin-2/NGAL, and measured 7526.4 pg/mL.

Human peripheral blood mononuclear cells (PBMCs) (1×10^6 cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. Cells were stimulated with 20 μ g/mL of PHA for 5 days. Aliquots of the culture supernates were removed, assayed for levels of human Lipocalin-2/NGAL, and measured 61.5 pg/mL.

MCF-7 (1×10^6 cells/mL) were cultured in DMEM/F12 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. Cells were cultured for 48h. Aliquots of the culture supernates were removed, assayed for levels of human Lipocalin-2/NGAL, and measured 72.3 pg/mL.

Serum - Eight human serum samples were evaluated for the presence of human Lipocalin-2/NGAL in this assay. All samples measured ranged from 38.2 to 84.1 ng/mL with an average of 64.3 ng/mL.

G. SPECIFICITY

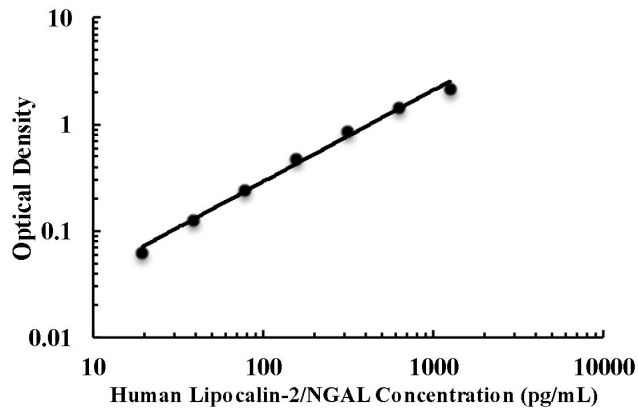
The following factors prepared at 50 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:	Other recombinants:
Lipocalin-1	mouse Lipocalin-2
MMP-9	rat Lipocalin-2

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	0.038 0.038	0.038	-
19.5	0.098 0.105	0.102	0.064
39.1	0.161 0.169	0.165	0.127
78.1	0.273 0.293	0.283	0.245
156.3	0.503 0.531	0.517	0.479
312.5	0.878 0.923	0.901	0.863
625	1.426 1.524	1.475	1.437
1250	2.154 2.234	2.194	2.156

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human Lipocalin-2/NGAL Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a rat antibody against human Lipocalin-2/NGAL.	1 plate
Human Lipocalin-2/NGAL Standard	Recombinant human Lipocalin-2/NGAL in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human Lipocalin-2/NGAL Detection Antibody	Biotinylated Lipocalin-2/NGAL antibody, lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Streptavidin-HRP A (200×)	200× Streptavidin conjugated to horseradish peroxidase.	1 vial
Calibrator Diluent Concentrate (2×)	A 2× concentrated buffered protein base used to dilute standard and samples.	1 vial
Detection Antibody Diluent Concentrate (10×)	A 10× concentrated buffered base used to dilute Detection Antibody.	1 vial
Reagent Diluent Concentrate (10×)	A 10× concentrated buffered base used to dilute Detection Antibody and HRP.	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
TMB Substrate	TMB ELISA Substrate Solution.	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Sealers	Adhesive strip.	3 strips

B. STORAGE

Unopened Kit	Store at 2-8 °C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Streptavidin-HRP A	May be stored for up to 1 month at 2-8 °C.*
	Diluted Wash Solution	
	TMB Substrate	
	Stop Solution	
	Standard	Prepare fresh for each assay. Standards may be stored for up to 1 month at -20°C*.
	Detection Antibody	Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer.*
	Calibrator Diluent Concentrate (2×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.
	Detection Antibody Diluent Concentrate (10×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Detection Antibody Diluent (1×). Prepare fresh for each assay.
	Reagent Diluent Concentrate (10×)	May be stored for up to 1 month at 2-8 °C.* Use and discard diluted Reagent Diluent (1×). Prepare fresh for each assay.
Microplate Wells	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.*	

* 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 $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1 \times).

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 \times g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles. Samples may require dilution with Calibrator Diluent (1 \times).

B. SAMPLE PREPARATION

Human serum samples recommend a 200-fold dilution. A suggested 200-fold dilution is 10 μL serum into a tube with 90 μL Calibrator Diluent (1 \times) to prepare a 10-fold diluted sample. Mix through and then pipette 10 μL of prepared 10-fold diluted sample into a tube with 190 μL Calibrator Diluent (1 \times) to prepare a final 200-fold diluted sample. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

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

Wash Buffer (1 \times) - 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 \times) into deionized or distilled water to prepare 500 mL of Wash Buffer (1 \times).

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

Detection Antibody Diluent (1 \times) - Use deionized or distilled water to prepare Detection Antibody Diluent (1 \times).

Reagent Diluent (1 \times) - Use deionized or distilled water to prepare Reagent Diluent (1 \times).

Detection Antibody (1 \times) - **Centrifuge briefly before opening. Reconstitution volume refer to vial label to prepare Detection Antibody (100 \times).** 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 \times) with Detection Antibody Diluent (1 \times). Prepare at least 15 minutes prior to use.

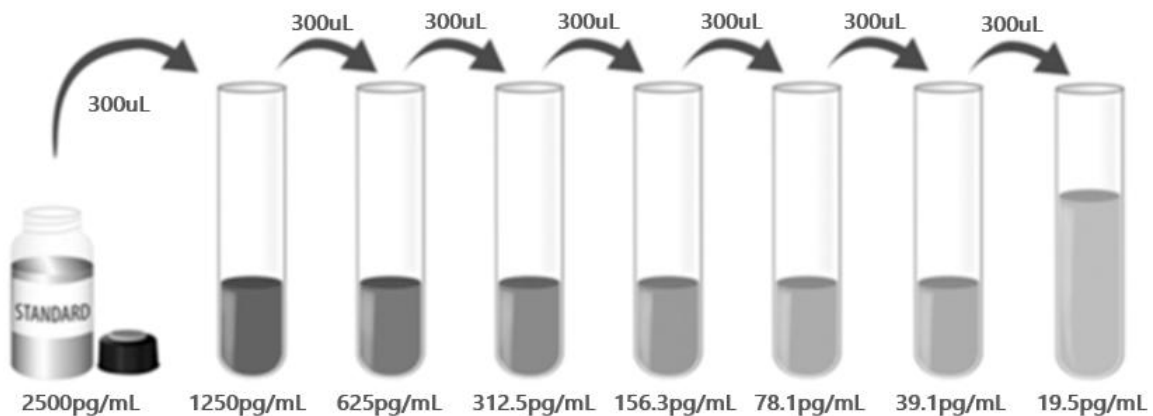
Streptavidin-HRP A (1 \times) - **Centrifuge briefly before opening.** Dilute to the working concentration specified on the vial label using Reagent Diluent (1 \times).

Human Lipocalin-2/NGAL Standard - **Centrifuge briefly before opening. Refer to**

the vial label for the reconstitution volume*. This reconstitution produces a stock solution of 2500 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 μ L of the appropriate Calibrator Diluent (1 \times) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1250 pg/mL standard serves as the high standard. The Calibrator Diluent (1 \times) 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 Solution protected from light. TMB Substrate Solution 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

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 μ L 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 Solution (400 μ L) 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 μ L of the Detection Antibody (1 \times) diluted in Detection Antibody Diluent (1 \times) to each well. Cover with a new adhesive strip and **incubate for 2 hours at room temperature.**
6. Repeat the aspiration/wash as in step 4.
7. Add 100 μ L of the working dilution of Streptavidin-HRP A to each well. Cover the plate and **incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 μ L of TMB Substrate to each well. **Incubate for 20 minutes at room temperature. Avoid placing the plate in direct light.**
10. Add 50 μ L 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 human Lipocalin-2/NGAL 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.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

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	A	B	C	D	E	F	G	H



产品信息及操作手册

人 Lipocalin-2/NGAL Valukine™ ELISA 试剂盒

目录号: **VAL183**

适用于定量检测天然和重组人 Lipocalin-2/NGAL 的浓度

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版本号 202309.2

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I. 背景

Lipocalin家族的成员具有有限的序列同一性，但具有高度保守的折叠，具有一个八股反平行 β 桶基序，其中包含一个内部配体结合位点(1, 2)。它们以携带类固醇激素、维生素、气味剂和代谢产物等小疏水分子的转运体而闻名(1-4)。Lipocalin-2，也称为中性粒细胞明胶酶相关脂脂素(NGAL)或铁钙素(Siderocalin)，最初被确定为中性粒细胞颗粒的组成部分(5)。从那时起，在大多数组织中观察到其表达，并在炎症期间由上皮细胞诱导其合成(4, 6)。Lipocalin-2与多种细胞过程有关，包括先天免疫应答、分化、肿瘤发生和细胞存活(4, 7-9)。它是一种25 kDa的蛋白质，以单体、同二聚体和异二聚体形式存在，后者与人基质金属蛋白酶9(MMP-9)相关(5)。它与MMP-9的结合可能通过保护MMP-9不被降解来调节蛋白酶活性(10)。小鼠同源基因(也称为24p3)在氨基酸水平上具有62%的序列同一性(4, 5)。

Lipocalin-2的功能仍有待阐明。研究表明，它能将细菌儿茶酚铁载体与铁离子结合(9, 11)。这表明Lipocalin-2可能通过结合细菌铁载体和限制细菌铁供应而起到抑菌剂的作用。这得到了观察结果的支持，即小鼠Lipocalin-2在toll样受体激活后在免疫细胞中被诱导，而Lipocalin-2小鼠敲除后抗细菌感染的能力下降(9, 12)。Lipocalin-2也可调节铁摄取进入哺乳动物细胞(8)。在肾脏中，Lipocalin-2介导的铁转运可能参与发育和保护肾损伤(8, 13-15)。Megalin, LDL受体家族的成员，和24p3 R被报道为Lipocalin-2的内吞受体(16, 17)。值得注意的是，Lipocalin-2对细胞的影响可能与环境有关。例如，它已被证明既是一种生存因子，也是一种促凋亡因子，并且促炎细胞因子对它的诱导在小鼠和人类之间可能有所不同(17-19)。

Lipocalin-2与几种病理过程有关。例如，与未受影响的对照皮肤相比，它在银屑病皮肤中上调，并且Lipocalin-2在贫血模型中抑制红细胞生成(20, 21)。Lipocalin-2在严重急性呼吸综合征(SARS)患者中升高，并可能作为急性肾损伤的生物标志物(15, 22)。它也与几种肿瘤类型有关，包括乳腺癌、卵巢癌、结直肠癌和胰腺癌(23-26)。它在癌症中的功能尚不清楚，尽管在乳腺癌和结肠癌模型中，肿瘤细胞的侵袭和转移行为被Lipocalin-2抑制(27, 28)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。大鼠抗人Lipocalin-2/NGAL 抗体包被于微孔板上，样品和标准品中的人Lipocalin-2/NGAL 会与固定在板上的抗体结合，游离的成分被洗去；接着加入生物素化的抗人Lipocalin-2/NGAL 检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶（Streptavidin-HRP）孵育。洗涤去除未结合的试剂后，加入TMB底物溶液（显色剂）。溶液颜色与结合的目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- ◆ 仅供科研使用，不可用于体外诊断；
- ◆ 该试剂盒适用于细胞培养上清样本和人血清样本；
- ◆ 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，在不同板中分别检测20次，以确定板间精确度。

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
Mean (pg/mL)	614.9	152.3	37.8	616.6	150.3	37.0
Standard Deviation	31.6	7.0	1.8	29.0	7.3	2.0
CV%	5.1	4.6	4.7	4.7	4.8	5.3

B. 回收率

在细胞培养基样本中掺入检测范围内不同水平的人Lipocalin-2/NGAL，测定其回收率。回收率范围在77.3-120.8%，平均回收率在98.7%。

C. 灵敏度

人Lipocalin-2/NGAL的最低可测量（MDD）一般小于2.79 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NS0表达的高纯度重组人Lipocalin-2/NGAL蛋白所校正。

E. 线性

不同的样本中含有或掺入高浓度的人Lipocalin-2/NGAL，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。

Dilution	Average % of Expected	Range (%)
1:2	99.9	84.2-114.7
1:4	102.7	78.9-119.9
1:8	104.8	84.2-123.7
1:16	96.6	74.5-121.0

F. 样本预值

细胞培养上清 - OCI-AML5 (1×10^6 cells/mL) 培养于含有20%胎牛血清和10 ng/mL重组人GM-CSF的 α -MEM培养基中，加100 U/mL青霉素和100 μ g/mL硫酸链霉素。培养48h后，得到的细胞培养上清分装检测人Lipocalin-2/NGAL含量，检测平均值为7526.4 pg/mL。

人外周血单核细胞(PBMCs) (1×10^6 cells/mL) 培养于含有10%胎牛血清的RPMI 1640培养基中，加100 U/mL青霉素和100 μ g/mL硫酸链霉素。细胞用20 μ g/mL的PHA刺激5天。得到的细胞培养上清分装检测人Lipocalin-2/NGAL含量，检测平均值为61.5 pg/mL。

MCF-7 (1×10^6 cells/mL) 培养于含有10%胎牛血清的DMEM/F12培养基中，加100 U/mL青霉素和100 μ g/mL硫酸链霉素。培养48h后，得到的细胞培养上清分装检测人Lipocalin-2/NGAL含量，检测平均值为72.3 pg/mL。

血清样本 - 使用本试剂盒检测了8份人血清样本中Lipocalin-2/NGAL的水平。8份样本的检测值范围为38.2-84.1 ng/mL，平均值为64.3 ng/mL。

G. 特异性

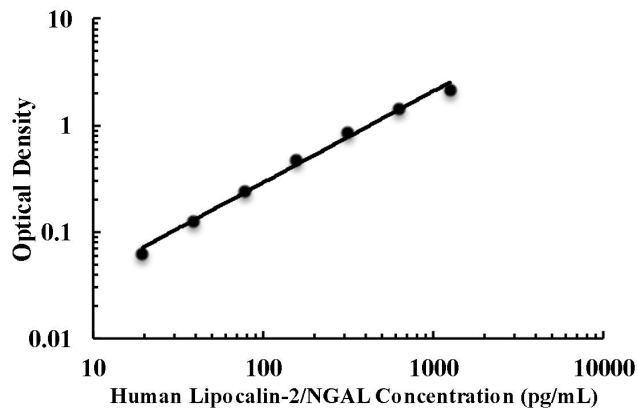
将以下因子配制成50 ng/mL的浓度来检测没有观察到明显的交叉反应或干扰。

Recombinant human:	Other recombinants:
Lipocalin-1	mouse Lipocalin-2
MMP-9	rat Lipocalin-2

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	OD	Average	Corrected
0	0.038 0.038	0.038	-
19.5	0.098 0.105	0.102	0.064
39.1	0.161 0.169	0.165	0.127
78.1	0.273 0.293	0.283	0.245
156.3	0.503 0.531	0.517	0.479
312.5	0.878 0.923	0.901	0.863
625	1.426 1.524	1.475	1.437
1250	2.154 2.234	2.194	2.156

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Human Lipocalin-2/NGAL Microplate	包被大鼠抗人 Lipocalin-2/NGAL 抗体的 96 孔聚苯乙烯板，8 孔×12 条	1 块板
Human Lipocalin-2/NGAL Standard	标准品（冻干粉），参考瓶身标签进行重溶	2 瓶
Human Lipocalin-2/NGAL Detection Antibody	生物素化的 Lipocalin-2/NGAL 检测抗体，冻干粉，参考瓶身标签进行重溶	1 瓶
Streptavidin-HRP A (200×)	200×浓缩的链霉亲和素标记的 HRP	1 瓶
Calibrator Diluent Concentrate (2×)	浓缩的标准品稀释液（2×）用于稀释标准品和样本	1 瓶
Detection Antibody Diluent Concentrate (10×)	浓缩的检测抗体稀释液（10×）用于稀释检测抗体	1 瓶
Reagent Diluent Concentrate (10×)	浓缩的试剂稀释液（10×）用于稀释检测抗体和 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	1 瓶
Stop Solution	终止液	1 瓶
Plate Sealers	封板膜	3 张

B. 试剂盒储存

未开封试剂盒	2-8℃储存；请在试剂盒有效期内使用	
已打开，稀释或重溶的试剂	链霉亲和素-HRP A	2-8℃储存，最多 30 天*
	洗涤缓冲液（1×）	
	TMB 底物溶液	
	终止液	
	标准品	使用时新鲜配制* 标准品-20℃储存，最多 30 天*
	检测抗体	分装， -20℃储存，最多 30 天*
	标准品稀释液（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
	检测抗体稀释液（10×）	2-8℃储存，最多 30 天* 请每次使用新鲜配制的 1×检测抗体稀释液，多余的丢弃
	试剂稀释液（10×）	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
包被的微孔板条	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*	

*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。样本可能需要用标准品稀释液（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。样本可能需要用标准品稀释液（1×）稀释。

B. 样本准备工作

血清样本建议用标准品稀释液（1×）200倍稀释后进行检测，例如：10 μL血清样本加到90 μL标准品稀释液（1×）中，充分混匀，即10倍稀释。然后取10 μL 10倍稀释后的样本加到190 μL标准品稀释液（1×）中，充分混匀，即制备成200倍稀释的样本。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浓缩洗涤液用去离子水或蒸馏水稀释配制成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稀释配制成标准品稀释液（1×）。

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

试剂稀释液（1×）：使用去离子水或蒸馏水稀释配制成试剂稀释液（1×）。

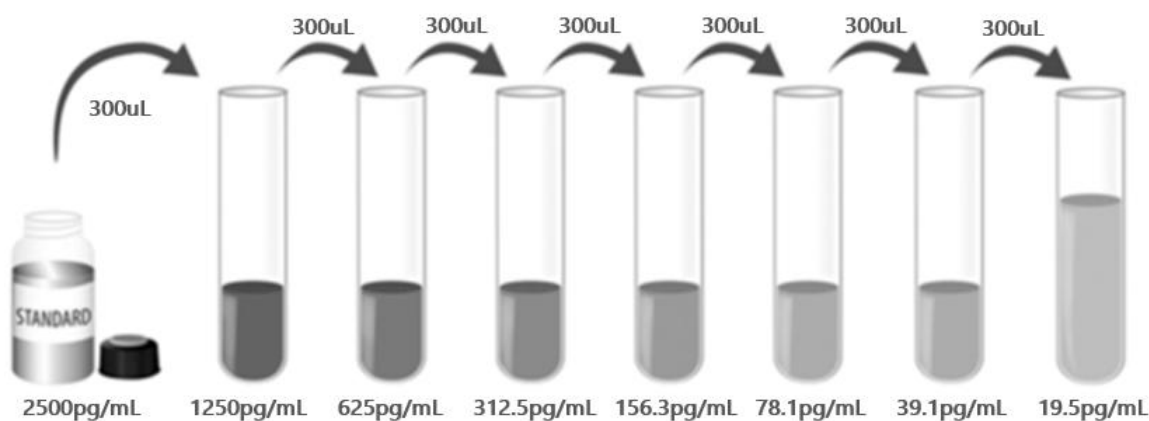
检测抗体（1×）：开盖前请瞬时离心。参考检测抗体瓶标签重溶冻干粉，制备检测抗体（100×）。轻轻震荡至少15分钟，其充分溶解。如有需要分装保存。用检测抗体稀释液（1×）稀释至检测抗体（1×），至少在使用前15分钟准备。

链霉亲和素-HRP A（1×）：开盖前请瞬时离心。用试剂稀释液（1×）将链霉亲和素-HRP A（200×）稀释至工作浓度。

人Lipocalin-2/NGAL 标准品：开盖前请瞬时离心。冻干标准品的重溶请参考瓶身标签。得到浓度为2500 pg/mL 标准品母液。轻轻震荡至少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 标准品稀释液 (1 \times)。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。1250 pg/mL 管作标准曲线最高点, 标准品稀释液 (1 \times) 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无色, 请避光保存; 加入微孔板后, TMB底物将由无色变成不同深度的蓝色;
- ◆ 终止液上板顺序应同显色底物上板顺序一致; 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 \times ）。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 A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。数据可以通过绘制人Lipocalin-2/NGAL浓度的对数与O.D.的对数来线性化，并且最佳拟合线可以通过回归分析来确定。该程序将产生足够但不太精确的数据拟合。

如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

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96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置

