PRODUCT INFORMATION & MANUAL

Mouse Proprotein Convertase 9/PCSK9 Valukine™ ELISA

Catalog Number: VAL633

For the quantitative determination of natural and recombinant mouse proprotein convertase 9/PCSK9 concentrations

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

Bio-Techne China Co. Ltd
P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001
info.cn@bio-techne.com

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

Version 202410.1

TABLE OF CONTENTS

I. BACKGROUND

PCSK9 (proprotein convertase subtilisin kexin 9), also called proprotein convertase 9 or NARC-1 (neural apoptosis-regulated convertase 1), is a member of the proteinase K subfamily of subtilisinrelated serine endoproteases. Mouse PCSK9 cDNA encodes 694 amino acids, including a signal peptide, a prodomain, and a catalytic domain. PCSK9 is highly expressed in the liver, intestine, and kidney. It is initially synthesized as a soluble 74 kDa precursor protein. In the endoplasmic reticulum, it undergoes autocatalytic intramolecular cleavage to generate a 14 kDa prodomain and a 60 kDa catalytic domain. While within the secretion pathway, the prodomain remains associated and functions as a chaperone for the catalytic domain (1-4). During secretion, a portion of active PCSK9 may undergo additional N-terminal proteolysis by furin or proprotein convertase 5/6A, creating an inactive 53 kDa form (5). This cleavage site is conserved between mouse and human or rat PCSK9, which share 78% or 93% amino acid sequence identity, respectively. While the 60 kDa protein is the major form, its ratio with the 53 kDa forms is variable in humans (5, 6).

The primary physiologic function of PCSK9 is to mediate the degradation of low density lipoprotein receptor (LDLR). Early observations indicated that gain-of-function missense mutations in the human PCSK9 gene can cause an autosomal dominant form of hypercholesterolemia (7, 8). The expression of PCSK9 is also upregulated by the sterol regulatory element binding proteins (SREBPs), a family of transcription factors that are responsible for the upregulation of genes involved in cholesterol and fatty acid metabolism, such as the LDLR gene (9, 10). Further experimental evidence revealed that when the mouse PCSK9 gene is deleted, LDLR expression in hepatocytes is increased. Conversely, PCSK9 over-expression decreases liver LDLR protein expression (11, 12). In humans, genetic analyses have shown that individuals who have nonsense or loss-of-function mutations in the PCSK9 gene have significantly lower plasma LDL cholesterol levels, while in mouse, administration of a PCSK9 neutralizing antibody or antisense oligonucleotides lowers serum cholesterol (1, 13-15). These investigations clearly indicate that PCSK9 plays a key role in reducing the hepatic LDLR levels. Paradoxically, administration of cholesterol-lowering drugs such as statins appear to enhance production of PCSK9 (6).

The underlying mechanism of cholesterol regulation by PCSK9 is as follows: under normal physiologic conditions, the LDLR is internalized at the cell surface and directed to the endosomes in order to be recycled back to the cell surface. PCSK9 binds to the EGF domain of the LDLR and prevents LDLR from being sorted to the endosomes. Instead, the PCSK9/LDLR complex is redistributed to the lysosomes for degradation (16-18). As such, PCSK9 regulates the amount of LDLR in the circulation and hence, modulates cholesterol levels. Serum PCSK9 concentrations have been found to be directly associated with cholesterol levels (19, 20). Since PCSK9 loss-of-function mutations strikingly reduce risk of coronary heart diseases, PCSK9 has become an attractive drug target (1, 21, 22). One approach is to generate small molecules that are able to interfere with PCSK9 autoactivation and its interaction with LDLR. Other approaches aiming to reduce the amount of PCSK9 in the circulation, such as small interfering RNAs (siRNAs), have also shown promise (23, 24).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for mouse PCSK9 has been pre-coated onto a microplate. Standards, control and samples are pipetted into the wells and any mouse PCSK9 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked antibody specific for mouse PCSK9 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 PCSK9 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 lysates.
- 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)	181	427	1427	213	469	1511
Standard Deviation	10.3	32.1	44.1	18.9	25.9	102
CV%	5.7	7.5	3.1	8.9	5.5	6.8

B. RECOVERY

The recovery of mouse PCSK9 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range (%)**
Cell culture media (n=6)	102	94-115
Tissue lysates* (n=5)	103	91-117

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

C. SENSITIVITY

Forty-five assays were evaluated and the minimum detectable dose (MDD) of mouse PCSK9 was less than 21.9 pg/mL. The mean MDD was 5.32 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-expressed recombinant mouse PCSK9 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 PCSK9 and diluted with Calibrator Diluent (1×) to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=6)	Tissue lysates (n=4)	Mouse serum* (n=4)	Mouse EDTA plasma* (n=4)	Mouse heparin plasma* (n=4)
1:2	Average % of Expected	98	101	100	98	97
	Range (%)	94-101	99-103	97-104	93-101	91-104
1:4	Average % of Expected	97	100	94	99	94
	Range (%)	91-100	90-104	93-95	99-104	87-99
1:8	Average % of Expected	97	101	94	101	92
	Range (%)	90-101	92-106	91-95	95-107	88-96
1:16	Average % of Expected	102	100	90	97	93
	Range (%)	93-106	90-107	85-94	92-101	91-95

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

F. SAMPLE VALUES

Mouse serum/plasma - Samples were evaluated for detectable levels of mouse PCSK9 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Mouse serum (n=20)	343	153-649	163
Mouse EDTA plasma (n=20)	337	166-612	118
Mouse heparin plasma (n=20)	324	141-565	100

Cell Culture Supernates - Organs from 2-3 mice were chopped into 1-2 mm pieces and cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin sulfate. The cell culture supernates were unstimulated or stimulated with 1.0 mg/mL of lipopolysaccharide for 1 or 2 days. An aliquot of the cell culture supernate was removed and assayed for levels of mouse PCSK9. Cell culture supernates from mouse brain, heart, lung, and spleen tissue measured below the low standard, 62.5 pg/mL. Cell culture supernates from liver and kidney tissue measured as follows.

Tissue Type	Observed Levels (pg/mL)
Kidney, (unstimulated for 1 day)	140
Liver, (unstimulated for 2 days)	889
Liver, (stimulated with 1.0 mg/mL for 2 days)	899

Tissue Lysates - Organs from 2-3 mice were rinsed with PBS to remove excess blood, chopped into 1-2 mm pieces, homogenized with a tissue homogenizer, and 1% v/v Triton™ X-100 was added. An aliquot of each tissue lysate was removed and assayed for levels of mouse PCSK9.

Tissue Type	Observed Levels (pg/mL)
Brain	1741
Heart	1603
Kidney	8953
Liver	36350
Lung	3898
Spleen	1800

G. SPECIFICITY

This assay recognizes natural and recombinant mouse PCSK9.

Mouse factors listed below were prepared at 50 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Human factors listed below were prepared at 500 ng/mL in Calibrator Diluent (1×) and assayed for cross-reactivity. Preparations of the following mouse factors (at 50 ng/mL) and human factors (at 500 ng/mL) in a mid-range mouse PCSK9 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:	**Recombinant human:**
LDLR	Furin
VLDLR	PCSK1
	PCSK7
	PCSK9

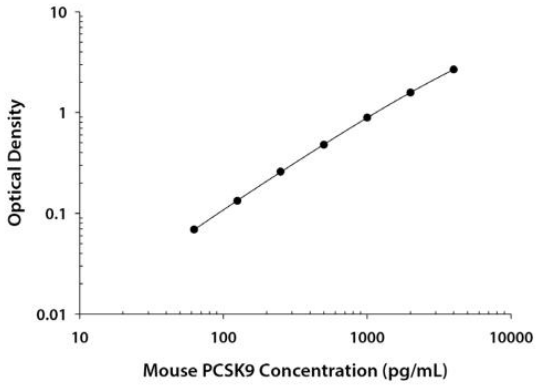
This assay detects 60 kDa, 53 kDa, and LDLR-complexed recombinant mouse PCSK9.

Rat serum was tested and found to be non-detectable in this assay.

IV. EXPERIMENT

EXAMPLE STANDARD

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



(pg/mL)	O.D.	Average	Corrected
0	0.016 0.017	0.017	—
62.5	0.085 0.086	0.086	0.069
125	0.149 0.150	0.150	0.133
250	0.275 0.277	0.276	0.259
500	0.494 0.497	0.496	0.479
1000	0.906 0.909	0.908	0.891
2000	1.584 1.607	1.596	1.579
4000	2.663 2.706	2.685	2.668

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Mouse PCSK9 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against mouse PCSK9.	1 plate
Mouse PCSK9 Conjugate	An antibody specific for mouse PCSK9 conjugated to horseradish peroxidase.	1 vial
Mouse PCSK9 Standard	Recombinant mouse PCSK9 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Mouse PCSK9 Control	Recombinant mouse PCSK9 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 RD1-21	A buffered protein base.	1 vial
Calibrator Diluent Concentrate (4×)/ RD5-26	A 4× 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 RD1-21</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.
- If using cell lysate samples, the following is also required: 1% v/v Triton™ X-100 in PBS.

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 Lysates - Cells must be lysed as directed in the Sample Values section before assaying. 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

Tissue lysate samples recommend a 10-fold dilution. A suggested 10-fold dilution is 40 µL of sample + 360 µL of Calibrator Diluent (1×). Optimal dilutions should be determined by the end user.

Mouse serum and plasma samples recommend a 200-fold dilution. A suggested 200-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent (1×). Complete the 200-fold dilution by adding 20 µL of the diluted sample + 380 µL of Calibrator Diluent. Optimal dilutions should be determined by the end user.

C. REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse PCSK9 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

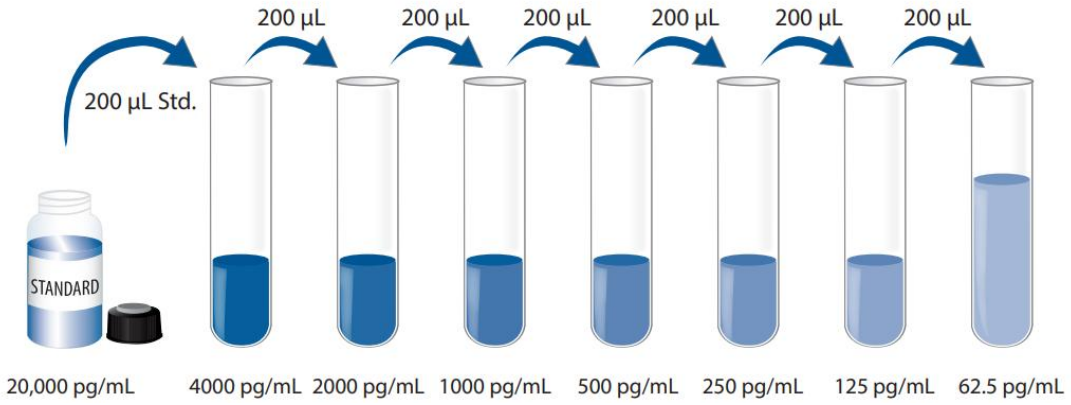
Wash Buffer (1×) - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer (1×).

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

Mouse PCSK9 Standard- Refer to the vial label for the reconstitution volume*
Reconstitute the Mouse PCSK9 Standard with Calibrator Diluent (1×). Do not substitute other diluents. This reconstitution produces a stock solution of 20000 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 800 µL of Calibrator Diluent (1×) into the 4000 pg/mL tube. Pipette 200 µL into each of the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard 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 RD1-21 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.** 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 PCSK9 Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours 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 PCSK9 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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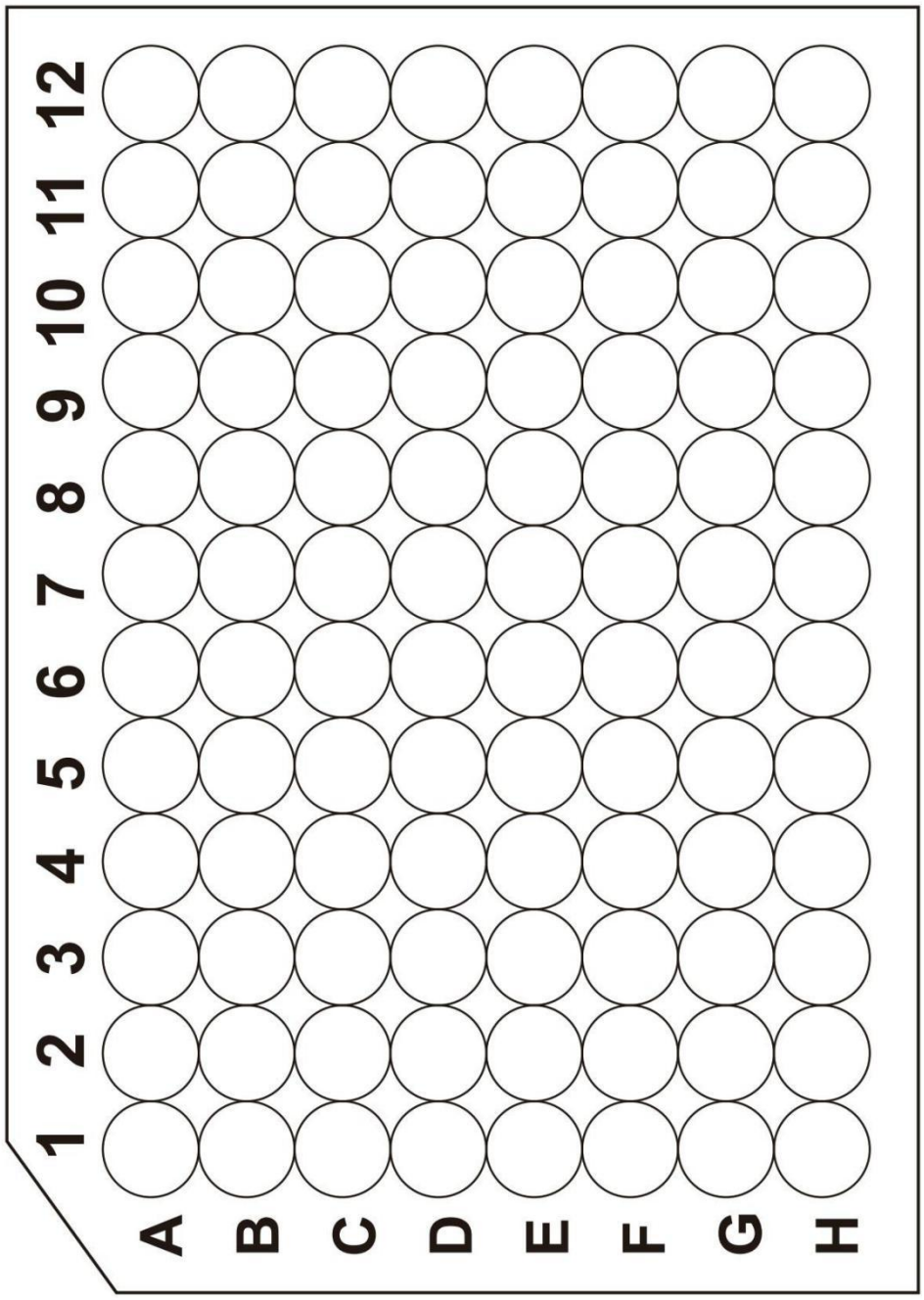
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

小鼠前蛋白转化酶 9/PCSK9 Valukine™ ELISA 试剂盒

目录号：VAL633

适用于定量检测天然和重组小鼠前蛋白转化酶 9/PCSK9 的浓度

科研专用，不可用于临床诊断

Bio-Techne China Co. Ltd

P: +86 (21) 52380373 P: 8009881270 F: +86 (21) 52381001

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版本号 202410.1

目录

I. 背景

PCSK9（前蛋白转化酶枯草溶菌素 9），也称为前蛋白转化酶 9 或 神经细胞凋亡调节转化酶 1（NARC-1），是枯草杆菌蛋白酶相关丝氨酸内切蛋白酶的蛋白酶 K 亚家族的成员。小鼠 PCSK9 cDNA 编码 694 个氨基酸，包括一个信号肽、一个前结构域和一个催化结构域。PCSK9 在肝脏、肠道和肾中高度表达。它最初合成为可溶性 74 kDa 的前体蛋白。在内质网状结构中，它经历自催化分子内切割形成了一个 14 kDa 的前结构域和一个 60 kDa 的催化结构域。在分泌途径中，前结构域保持关联并作为催化结构域的伴侣发挥作用(1-4)。在分泌过程中，一部分活化的 PCSK9 可能通过前蛋白转化酶 5/6A 进行额外的 N 末端蛋白水解从而形成非活性的 53 kDa 分子形式(5)。这个酶切位点在小鼠和人或者大鼠之间是保守的，分别具有 78%或 93%的氨基酸序列同源性(5, 6)。

PCSK9 的主要生理功能是介导 LDLR 的降解。早期观察表明，人类 PCSK9 基因的功能获得性错义突变可导致常染色体显性高胆固醇血症(7, 8)。PCSK9 的表达也被 SREBP 上调，SREBP 是一个转录因子家族，负责上调包括胆固醇和脂肪酸代谢的基因，例如 LDLR 基因(9, 10)。进一步实验证据表明，当小鼠 PCSK9 基因缺失时，LDLR 在肝细胞的表达增加。相反，PCSK9 过表达会降低肝脏 LDLR 蛋白的表达(11, 12)。在人类中，遗传分析表明，PCSK9 基因中具有无义或功能丧失突变的个体具有显着降低的血浆 LDL 胆固醇水平，而在小鼠中，注射 PCSK9 中和抗体或反义寡核苷酸可降低血清胆固醇(1, 13-15)。这些调查清楚地表明，PCSK9 在降低肝脏 LDLR 水平中起关键作用。矛盾的是，服用他汀类降胆固醇药物似乎可以增强 PCSK9 的产生(6)。

PCSK9 调节胆固醇的潜在机制如下：正常生理条件时，LDLR 在细胞表面内化到细胞中以便其再次回至细胞表面。PCSK9 与 LDLR 的 EGF 结构域结合，并阻止 LDLR 内化到胞内。相反，PCSK9/LDLR 复合物是重新分布到溶酶体进行降解(16-18)。因此，PCSK9 调节循环中 LDLR 的量，从而调节胆固醇水平。血清中 PCSK9 的浓度被发现与胆固醇水平直接相关(19, 20)。由于 PCSK9 功能丧失突变显著降低了冠心病的风险，因此 PCSK9 已成为一种有吸引力的药物目标(1, 21, 22).。一种方法是合成一种能够干扰 PCSK9 自激活和干扰其与 LDLR 相互作用的小分子。其他方法旨在减少循环中 PCSK9 的含量，例如 siRNA，也显示出较好的前景(23, 24)。

II. 概述

A. 检测原理

本实验采用双抗体夹心ELISA法。抗小鼠PCSK9抗体包被于微孔板上，样品，质控品和标准品中的小鼠PCSK9会与固定在板上的抗体结合，游离的成分被洗去；加入过氧化酶标记的抗小鼠PCSK9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裂解物样本；
- 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)	181	427	1427	213	469	1511
标准差	10.3	32.1	44.1	18.9	25.9	102
CV%	5.7	7.5	3.1	8.9	5.5	6.8

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PCSK9，测定其回收率。

样本类型	平均回收率%	范围 (%)
细胞培养基 (n=6)	102	94-115
组织裂解物* (n=5)	103	91-117

*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

45次检测结果表明，小鼠PCSK9的最低可测剂量（MDD）低于21.9 pg/mL。平均MDD为5.32 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表达的重组小鼠PCSK9校正。

E. 线性

不同的样本中含有或掺入高浓度的小鼠PCSK9，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=6)	组织裂解物* (n=4)	小鼠血清* (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（%）	98	101	100	98	97
	范围（%）	94-101	99-103	97-104	93-101	91-104
1:4	平均值/期待值（%）	97	100	94	99	94
	范围（%）	91-100	90-104	93-95	99-104	87-99
1:8	平均值/期待值（%）	97	101	94	101	92
	范围（%）	90-101	92-106	91-95	95-107	88-96
1:16	平均值/期待值（%）	102	100	90	97	93
	范围（%）	93-106	90-107	85-94	92-101	91-95

*样品在测定前按照样品制备部分的指示进行稀释

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PCSK9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)	343	153-649	163
小鼠EDTA血浆样本 (n=20)	337	166-612	118
小鼠肝素血浆样本 (n=20)	324	141-565	100

细胞上清样本 - 将 2-3 只小鼠的器官切成 1-2 mm 的小块，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 mg/mL 硫酸链霉素的 RPMI培养基中。细胞培养上清不刺激或用 1.0 mg/mL 脂多糖刺激 1 或 2 天。取出等量的细胞培养上清，检测小鼠 PCSK9 的水平。小鼠脑、心、肺和脾组织的细胞培养上清液的检测值低于最低标准，62.5 pg/mL。肝脏和肾脏组织的细胞培养上清测定结果如下。

组织类型	**检测值 (pg/mL)**
肾脏, (不刺激1天)	140
肝脏, (不刺激2天)	889
肝脏, (1.0 mg/mL LPS 刺激2天)	899

组织裂解物样本 - 用 PBS 冲洗 2-3 只小鼠的器官以去除多余的血液，切成 1-2 mm 的小块，用组织匀浆器匀浆，然后加入 1% v/v Triton™ X-100。取出等量的组织裂解物，检测小鼠 PCSK9 的水平。

组织类型	**观测水平 (pg/mL)**
大脑	1741
心脏	1603
肾脏	8953
肝脏	36350
肺部	3898
脾	1800

G. 特异性

此ELISA法可检测天然及重组小鼠PCSK9。

将以下表小鼠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PCSK9的交叉反应。将以下表人类因子用标准品稀释液（1×）配制成500 ng/mL的浓度来检测与小鼠PCSK9的交叉反应。将下表小鼠因子（50 ng/mL）和人类因子（500 ng/mL）掺入中间范围的重组小鼠PCSK9对照品中，来检测对小鼠PCSK9的干扰。未观察到明显的交叉反应或干扰

Recombinant mouse:	**Recombinant human:**
LDLR	Furin
VLDLR	PCSK1
	PCSK7
	PCSK9

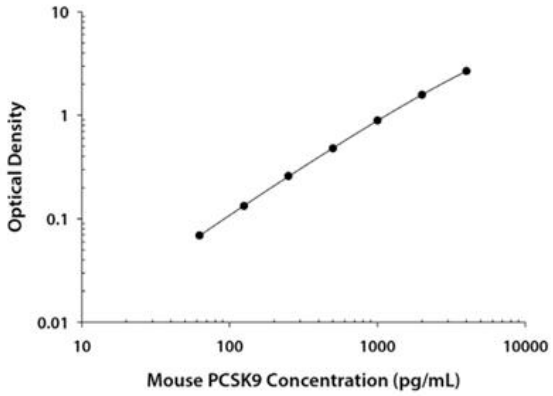
本试剂盒可检测 60 kDa、53 kDa 和 LDLR 复合物重组小鼠 PCSK9。

本试剂盒不能检测大鼠血清样本。

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。



(pg/mL)	O.D.	Average	Corrected
0	0.016 0.017	0.017	—
62.5	0.085 0.086	0.086	0.069
125	0.149 0.150	0.150	0.133
250	0.275 0.277	0.276	0.259
500	0.494 0.497	0.496	0.479
1000	0.906 0.909	0.908	0.891
2000	1.584 1.607	1.596	1.579
4000	2.663 2.706	2.685	2.668

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
Mouse PCSK9 Microplate	包被抗小鼠PCSK9抗体的96孔聚苯乙烯板，8孔× 12条	1块板
Mouse PCSK9 Conjugate	酶标检测抗小鼠PCSK9抗体	1瓶
Mouse PCSK9 Standard	小鼠PCSK9标准品（冻干），参考瓶身标签进行重溶	1瓶
Mouse PCSK9 Control	小鼠PCSK9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 RD1-21	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	1瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1瓶
TMB Substrate	TMB ELISA底物溶液/TMB底物溶液	2瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8°C储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="8">已打开，稀释或重溶的试剂</td><td>洗涤液（1×）</td><td rowspan="6">2-8°C储存，最多30天*</td></tr>
<tr><td>检测液RD1-21</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°C 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 °C储存，最多30天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量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
- 100 mL和500 mL量筒
- 用于稀释标准品和样品的管子
- 如果使用细胞裂解物样本，还需要以下材料： 含有1% v/v Triton™ X-100的PBS。

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。

组织裂解物：在检测前样本必须按照样本值部分进行裂解。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。

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°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：*本试剂盒对枸橼酸钠血浆尚未被验证。*

B. 样本准备工作

组织裂解物样本建议用标准品稀释液（1×）10倍稀释后进行检测，即40 µL样品+360 µL标准品稀释液（1×）。最佳稀释度应由最终用户确定。

小鼠血清样本和血浆样本建议用标准品稀释液（1×）200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+380µL标准品稀释液（1×），即是200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PCSK9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。

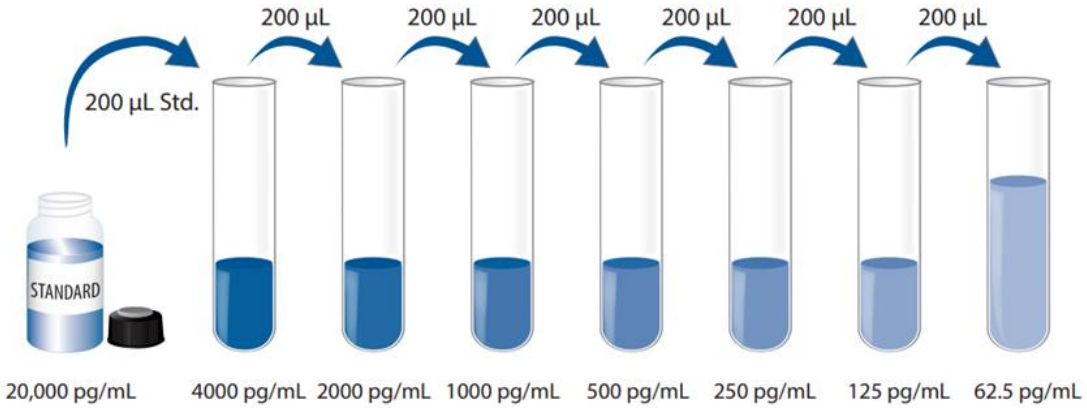
洗涤液（1×）：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将20 mL浓缩洗涤液（25×）用蒸馏水或去离子水稀释配制成500 mL工作浓度的洗涤液（1×）。

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PCSK9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 PCSK9标准品。不得使用其他试剂。得到浓度为20000 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。

加入800 µL标准品稀释液（1×）到 4000pg/mL管中。剩余每管中加入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。4000 pg/mL作标准曲线最高点，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RD1-21。
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PCSK9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PCSK9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