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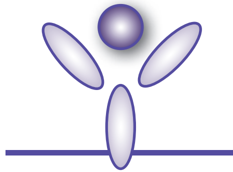
The path to legendary discovery™

LEGEND MAX™

ELISA Kit with Pre-coated Plates



Mouse PCSK9

Cat. No. 443207

ELISA Kit for Accurate Quantitation of Mouse PCSK9 from Serum, Plasma, Cell Culture Supernatant, Cell Lysate, Tissue Homogenate, and Other Biological Fluids

BioLegend, Inc.
biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

For Research Purposes Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of BioLegend is strictly prohibited.

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Introduction:

Protein Convertase Subtilisin/Kexin Type 9 (PCSK9) belongs to the proteinase K subfamily of subtilases and is expressed as a soluble zymogen that undergoes autocatalytic intramolecular processing in the endoplasmic reticulum. Mouse PCSK9 is a 694 amino acid glycoprotein and shares 78% and 92% amino acid identity with human and rat PCSK9, respectively.

PCSK9 is mainly expressed in liver, and at lower levels in kidney, intestine, and brain. It regulates plasma cholesterol homeostasis via binding to low-density lipid receptor family members such as Low-density lipoprotein receptor (LDLR). The binding of PCSK9 to LDLR enhances the degradation of the LDLR in endosomes/lysosomes, resulting in increased circulating LDL-Cholesterol (LDL-C). Elevated LDL-C levels are a major risk factor for cardiovascular disease and atherosclerosis. Individuals with genetic loss-of-function mutations in *PCSK9* have reduced plasma levels of LDL-C and are protected from coronary heart disease. Thus, PCSK9 has gained attention as a pharmacological target for cardiovascular diseases and atherosclerosis.

BioLegend's LEGEND MAX™ Mouse PCSK9 ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a rat anti-mouse PCSK9 monoclonal antibody. The Detection Antibody is a Biotinylated goat anti-mouse PCSK9 polyclonal antibody. This kit is specifically designed for the accurate quantitation of mouse PCSK9 from serum, plasma, cell culture supernatant, cell lysate, tissue homogenate, and other biological fluids. It is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Anti-Mouse PCSK9 Pre-coated 96-well Strip Microplate	1 plate		76165
Mouse PCSK9 Detection Antibody	1 bottle	12 mL	76166
Mouse PCSK9 Standard	1 vial	lyophilized	76168
Avidin-HRP	1 bottle	12 mL	77897
Assay Buffer A	1 bottle	25 mL	78232
Wash Buffer (20X)	1 bottle	50 mL	78233
Substrate Solution D	1 bottle	12 mL	78115
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	4 sheets		78101

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Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

<table>
<tr><th colspan="2">Opened or Reconstituted Components</th></tr>
<tr><td>Microplate Strips</td><td>If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.</td></tr>
<tr><td>Standard</td><td>The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Detection Antibody</td><td rowspan="6">Store opened reagents between 2°C and 8°C and use within one month.</td></tr>
<tr><td>Avidin-HRP</td></tr>
<tr><td>Assay Buffer A</td></tr>
<tr><td>Wash Buffer (20X)</td></tr>
<tr><td>Substrate Solution D</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend’s website for details (www.biolegend.com/msds).
2. Substrate Solution D is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.

3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
4. Stop Solution contains strong acid and is corrosive. *Wear eye, hand, and face protection to handle and follow state or county regulation to dispose.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant and Cell Lysate: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x *g*. Remove serum layer. *Assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.*

Plasma: Collect blood samples in heparin, citrate or EDTA-containing tubes. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. *Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles*.

Tissue Homogenates: Using an appropriately sized homogenizer, homogenate mouse liver and kidneys into 1 mL per organ of cold 1XPBS containing protease inhibitors. After two rounds of freeze-thaw cycles, centrifuge tissue homogenates, and collect supernatant. *Assay immediately or store samples at < -70°C. Avoid repeated freeze-thaw cycles.*

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Mouse PCSK9 Standard by adding the volume of Assay Buffer A to make the 20 ng/mL standard stock solution (Refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/LEGEND MAX Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15 minutes, then briefly vortex to mix completely.

3. For measuring serum or plasma samples, a 100-fold dilution is recommended using Assay Buffer A. For example, dilute 3 µL of sample in 297 µL of Assay Buffer A.

4. For cell culture supernatant, cell lysate and tissue homogenate samples, dilutions need to be determined individually in a preliminary experiment. If dilutions are necessary, samples should be diluted in the corresponding cell culture medium, cell lysate buffer or tissue homogenate buffer.

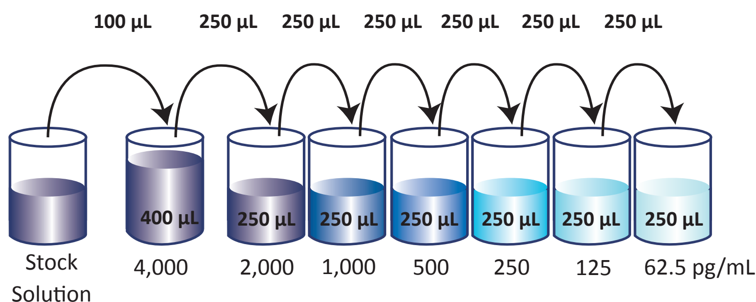
Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.

2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.

3. Prepare 500 µL of the 4,000 pg/mL top standard by diluting 100 µL of the standard stock solution in 400 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 4,000 pg/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the mouse PCSK9 standard concentrations in the tubes are 4,000 pg/mL, 2,000 pg/mL, 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, respectively. Assay Buffer A serves as the zero standard (0 pg/mL).



4. Add 50 µL of Assay Buffer A to each well that will contain either standard dilutions or samples.

5. Add 50 µL of standard dilutions or properly diluted samples to the appropriate wells.

6. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.
7. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer. Wash the plate with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping plate upside down on absorbent paper. All subsequent washes should be performed similarly.
8. Add 100 µL of Mouse PCSK9 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.
9. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 7.
10. Add 100 µL of Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.
11. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 7. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
12. Add 100 µL of Substrate Solution D to each well and incubate at room temperature for 15 minutes in the dark. Wells containing mouse PCSK9 should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
13. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.
14. Read absorbance at 450 nm immediately. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

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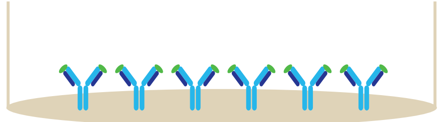
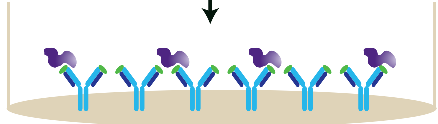
Assay Procedure Summary

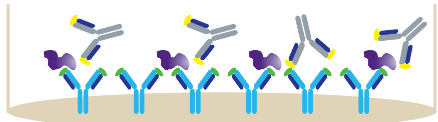
1. Add 50 µL Assay Buffer A



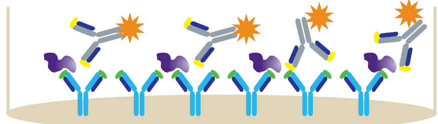
2. Add 50 µL diluted standards or samples
Incubate 2 hrs, RT, shaking



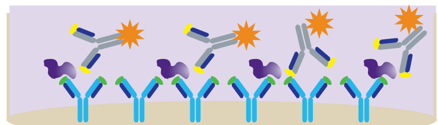
3. Wash 4 times
Add 100 µL Detection Antibody solution
Incubate 1 hr, RT, shaking



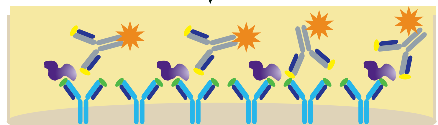
4. Wash 4 times
Add 100 µL Avidin-HRP solution
Incubate 30 mins, RT, shaking



5. Wash 5 times
Add 100 µL Substrate Solution D
Incubate 15 mins, RT, in the dark



6. Add 100 uL Stop Solution



7. Read absorbance at 450 nm and 570 nm

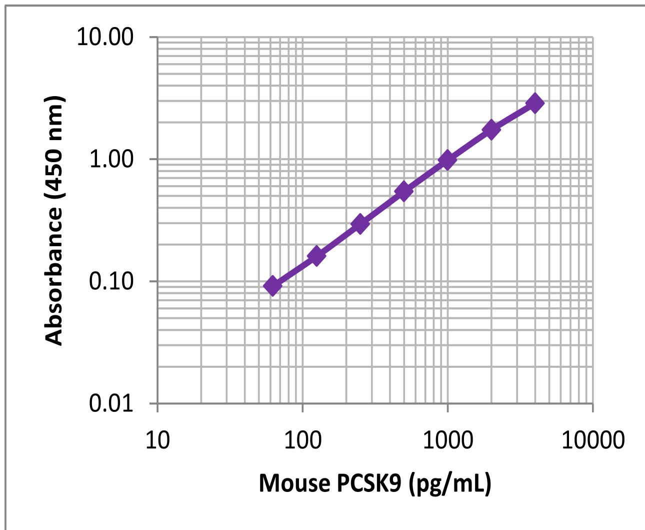
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with protein concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown protein concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the protein concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity: No cross-reactivity was observed with the following recombinant proteins: mouse LDLR, mouse Furin, human PCSK9, human Furin and human LDL, each at 50 ng/mL.

Sensitivity: The average minimum detectable concentration is 8.2 pg/mL (n=10).

Linearity: Mouse samples were diluted with Assay Buffer A to produce samples with values within the dynamic range, and then assayed with the kit to determine the dilution linearity up to 8-fold dilutions.

Sample Type	N	% Linearity
Serum	4	89
Plasma (Heparin, Citrate, EDTA)	12	
F9 cell culture media	1	103
F9 cell lysate	1	87
Mouse liver homogenate	1	105

Recovery: Recombinant mouse PCSK9, at concentrations of 2, 0.5, and 0.13 ng/mL, was spiked into the following mouse samples, then analyzed with the LEGEND MAX™ Mouse PCSK9 ELISA Kit.

Sample Type	N	% Recovery
Serum*	2	93
Plasma (Heparin, EDTA)*	4	
Citrate plasma*	2	59
F9 cell culture media*	1	88
F9 cell lysate*	1	99

**Samples were diluted prior to spiking.*

Intra-Assay Precision: Two samples with different recombinant mouse PCSK9 concentrations were tested with 16 replicates in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	4,258.4	481.9
Standard Deviation	214.6	30.4
% CV	5.0	6.3

Inter-Assay Precision: Two samples with different concentrations of recombinant mouse PCSK9 were assayed in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assay	4	4
Mean Concentration (pg/mL)	1,337.3	130.3
Standard Deviation	157.4	10.6
% CV	11.8	8.1

Biological Samples:

Serum or Heparin Plasma

Serum or Heparin plasma samples of the following strains were pooled from a minimum of ten mice each and tested for endogenous PCSK9. The concentrations (ng/mL) measured are shown below:

Mouse strain	Serum	Heparin plasma	EDTA plasma	Citrate plasma
BALB/c	26.6	37.4	17.9	21.3
C57BL/6	61.6	60.2	40.1	32.5
Swiss Webster	201.0	231.0	75.9	85.7
CD-1	>400.0	116.0	86.4	60.9

Cell Culture Supernatants

Mouse splenocytes or kidney cells ($1x10^6$/mL) were either unstimulated or stimulated with LPS (1 μg/mL). F9 cells were incubated without any stimulation. All culture supernatants with/without stimulation were removed on day 3. Mouse PCSK9 levels (pg/mL) were assayed as below.

Sample	Treatment	Day	Concentration (pg/mL)
F9	Unstimulated	d3	10,525
Splenocytes	Unstimulated	d3	ND*
	LPS stimulated	d3	190.0
Kidney	Unstimulated	d3	371.3
	LPS stimulated	d3	459.1

*ND: Not detectable

Cell Lysates

F9 cells were incubated in DMEM media for three days. Cells were rinsed with cold PBS and lysed with RIPA cell lysis buffer (0.75% NP-40, 0.25% Nadeoxycholate acid and 0.05% SDS). Cell lysate was freeze-thawed once and centrifuged for 5 minutes at 5,000 x *g*. Supernatant was harvested and assayed for the level of PCSK9. The concentration measured was 1.4 ng/mL.

Tissue Homogenates

Liver and kidneys were isolated from a BALB/c mouse (female, <6 months old) and rinsed with cold 1X PBS to remove excess blood. One milliliter of 1XPBS was added to each organ, and tissue was homogenized with a tissue homogenizer. After two freeze-thaw cycles, homogenates were centrifuged for 5 minutes at 5,000 x *g*. Supernatant was harvested and assayed for PCSK9 level as below.

Sample	Concentration (ng/mL)
Liver Homogenates	28.4
Kidney Homogenates	4.4

Detection of Complexed PCSK9:

To detect PCSK9 in complexed forms, recombinant mouse PCSK9 (400 ng/mL) was mixed with either recombinant LDLR or highly purified native LDL at the indicated molar ratios (1:1 ratio for LDLR and LDL is 400 ng/mL and 1 mg/mL, respectively). The protein mixtures were incubated at 37°C for overnight. The levels of PCSK9 in the mixture were measured as below.

PCSK9 : LDLR (Molar ratio)	PCSK9 (ng/mL)	Detection (%)
1 : 0	458	100
1 : 1	438	96
1 : 10	371	81

PCSK9 : LDL (Molar ratio)	Sample (ng/mL)	Detection (%)
1 : 0	380	100
1 : 0.05	342	90
1 : 0.1	294	77
1 : 0.5	92	24
1 : 1	46	12
1 : 2	32	9

Troubleshooting Guide

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN_3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

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Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

LEGEND MAX™ Kits are manufactured by **BioLegend Inc.**
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