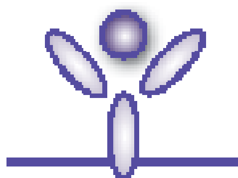
LEGEND MAX™

ELISA Kit



Human PCSK9

Cat. No. 443107

ELISA Kit for Accurate Quantitation of Human PCSK9 from Cell Culture Supernatant, Serum, Plasma, 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.

Table of Contents   Page

Introduction... 2

Materials Provided... 2

Materials to be Provided by the End-User.................................... 3

Storage Information.. 3

Health Hazard Warnings.. 4

Specimen Collection and Handling.. 4

Reagent and Sample Preparation.. 4

Assay Procedure... 5

Assay Procedure Summary.. 7

Calculation of Results.. 8

Typical Data... 8

Performance Characteristics.. 9

- Specificity.. 9
- Sensitivity.. 9
- Recovery.. 9
- Linearity... 9
- Intra-Assay Precision.. 10
- Inter-Assay Precision.. 10
- Biological Samples... 10

Troubleshooting Guide.. 11

ELISA Plate Template.. 13

Introduction:

Proprotein convertase subtilisin/kexin 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. It is a 692 amino acid (aa) glycoprotein with an overall domain structure similar to other proprotein convertase members. It is comprised of a signal peptide (aa 1–30), a pro segment (aa 31–152), a catalytic domain (aa 153–407), a hinge region (aa 408–452), and a C-terminal Cys-His-rich domain (aa 453–692).

The biological roles of PCSK9 are closely related to its binding partners, low-density lipoprotein (LDL) and LDL receptor (LDLR). At the cell surface, secreted PCSK9 binds at neutral pH to the EGF-A-like repeat of the LDLR via its catalytic domain. The binding of PCSK9 to LDLR enhances the degradation of the LDLR in endosomes/lysosomes, resulting in increased circulating LDL-cholesterol (LDL-C). Thus, PCSK9 has gained attention as a pharmacological target for cardiovascular diseases and atherosclerosis. It also binds to the Apolipoprotein B (ApoB) in LDL, although its role in this complex is not clearly understood. Due to the interaction of PCSK9 with LDLR, the levels of free PCSK9 or its complexed forms, are important indicators for measuring risk of cardiovascular diseases or response to a therapy.

The LEGEND MAX™ Human PCSK9 ELISA kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a rat monoclonal anti human PCSK9 antibody. The Detection Antibody is a biotinylated goat polyclonal anti-human PCSK9 antibody. This kit is specifically designed for the accurate quantitation of human PCSK9, both free and complexed form-with LDLR or LDL, in cell culture supernatant, serum, plasma and other biological fluids. This kit is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume (per bottle)	Part #
Anti-Human PCSK9 Pre-coated 96 well Strip Microplate	1 plate		76157
Human PCSK9 Detection Antibody	1 bottle	12 mL	76158
Human PCSK9 Standard	1 vial	lyophilized	76160
Avidin-HRP	1 bottle	12 mL	77897
Assay Buffer A	1 bottle	25 mL	78232
Assay Diluent D	1 bottle	25 mL	76384
Wash Buffer (20X)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	79132
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	4 sheets		78101

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 wells</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="7">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>Assay Diluent D</td></tr>
<tr><td>Wash Buffer (20X)</td></tr>
<tr><td>Substrate Solution F</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 F 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. *Wear eye, hand, and face protection.*
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: 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, EDTA, or Citrate-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*.

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 mix until dissolved.

2. Reconstitute the lyophilized Human PCSK9 Standard by adding the volume of Assay Diluent D to make the 200 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. The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C.

3. It is recommended that cell culture supernatant be diluted 1:5 with Assay Diluent D 15 minutes prior to analysis, and used within two hours after dilution. For example, 40 µL of cell culture supernatant is diluted into 160 µL of Assay Diluent D to make a 5-fold dilution. Plasma and serum samples should be diluted 1:20 with Assay Diluent D 15 minutes prior to analysis, and used within two hours after dilution. For example, 10 µL of a plasma sample is diluted into 190 µL of Assay Diluent D to make a 20-fold dilution. If further dilution is needed, samples should be diluted with Assay Diluent D.

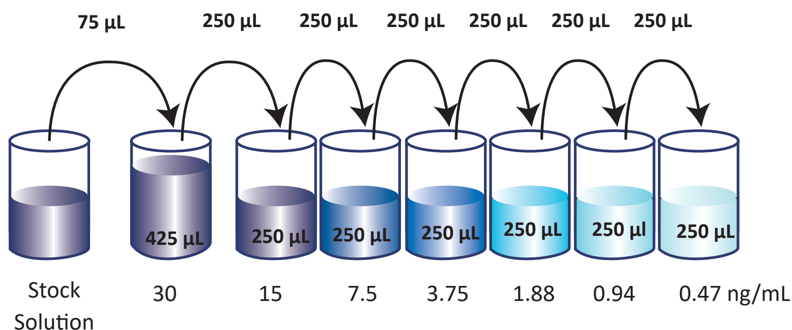
Assay Procedure:

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. A standard curve is required for each assay.

2. LEGEND MAX™ Human PCSK9 kit's performance is optimum when the plate is incubated on a shaker at higher than 200 rpm during the experiment except Substrate Solution F incubation. For a circumstance without a shaker, it is recommended that the plate with standards and samples are incubated at between 2 and 8 °C overnight for the optimum performance.

3. Transfer 75 µL of the reconstituted Human PCSK9 solution at 200 ng/mL to a tube containing 425 µL Assay Diluent D to make the 30 ng/mL top standard. Perform six two-fold serial dilutions of the 30 ng/mL top standard in separate tubes using Assay Diluent D as the diluent. Thus, the human PCSK9 standard concentrations in the tubes are 30 ng/mL, 15 ng/mL, 7.5 ng/mL, 3.75 ng/mL, 1.88 ng/mL, 0.94 ng/mL, and 0.47 ng/mL, respectively. Assay Diluent D serves as the zero standard (0 ng/mL).



4. Wash the plate 4 times with at least 300 µl of 1X Wash Buffer per well and blot residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.

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

6. Add 50 µL of standard dilutions or samples to the appropriate wells.

7. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at higher than 200 rpm. Without a shaker, overnight incubation at 2 to 8 °C is recommended.

8. Discard the plate contents into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

9. Add 100 µL of Human PCSK9 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.

10. Discard the plate contents into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

11. Add 100 µL of Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.

12. Discard the contents of the plate into a sink, then wash the plate 6 times with 1X Wash Buffer. Soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash to minimize background.

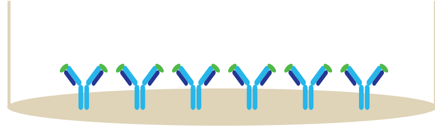
13. Add 100 µL of Substrate Solution F to each well and incubate for 15 minutes in the dark. Wells containing human PCSK9 should turn blue in color with intensity proportional to concentration. It is not necessary to seal the plate during this step.

14. Stop the reaction by adding 100 µL of Stop Solution to each well. The well color should change from blue to yellow.

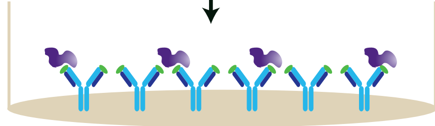
15. Read absorbance at 450 nm within 20 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

LEGEND MAX™ Human PCSK9 ELISA Kit

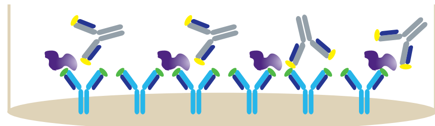
1. Wash 4 times
   Add 50 µL Assay Buffer A



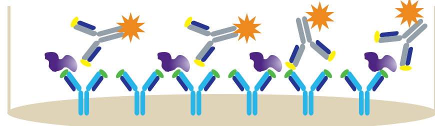
2. Add 50 µL diluted standards or samples Incubate 2 hrs, RT, shaking



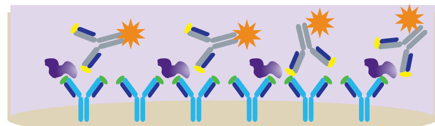
3. Wash 4 times
   Add 100 µL Detection Antibody solution
   Incubate 1 hr, RT, shaking



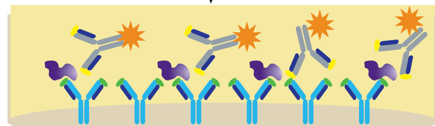
4. Wash 4 times
   Add 100 µL Avidin-HRP solution
   Incubate 30 mins, RT, shaking



5. Wash 6 times
   Add 100 µL Substrate Solution F
   Incubate 15 mins, RT, in the dark



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

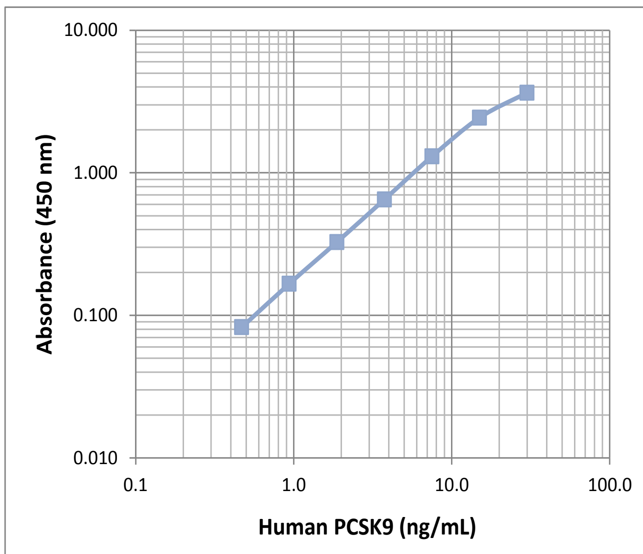
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If 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 antigen concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown antigen 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 PCSK9 concentration.

Because 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 when this kit was used to analyze human furin, human LDLR, mouse furin, and mouse PCSK9, each at 1 μg/mL.

Measurement of human PCSK9 in its complexes: LDLR and LDL are known to form complexes with PCSK9. Human PCSK9 levels were measured from commercial recombinant human LDLR/PCSK9 complex and prepared LDL/PCSK9 complex. In order to evaluate the kit performance on complexes, linearity of the detected values and the ratio between the detected values and the expected values were analyzed and compared with a competitor's kit. The linearity of each sample was tested between 20- and 160-fold dilution.

Kit	Competitor		LEGEND MAX™ Kit	
Complexing Molecule	LDLR	LDL	LDLR	LDL
Average % Linearity	103	97	96	87
Expected Value (ng/mL)	510	400	510	400
Average Detected Value (ng/mL)	53	89	494	344
Detected / Expected Value (%)	10.4	22.3	96.7	86.0

Sensitivity: The average minimum detectable concentration of human PCSK9 is 0.125 ng/mL.

Recovery: The recovery of human PCSK9 spiked to three levels throughout the range of the assay in plasma and serum was evaluated.

	Serum	EDTA	Heparin	Citrate
Number of Samples	5	4	4	5
Average % Recovery	98	97	97	97
Range (%)	94-102	90-107	84-107	96-99

Linearity: Five paired human serum and plasma samples were diluted with Assay Diluent D at 20- to 160-fold to produce sample concentrations within the dynamic range of the assay.

	Serum	EDTA	Heparin	Citrate
Number of Samples	5	5	5	5
Average % of Expected	97	87	104	99
Range (%)	92-102	81-91	99-107	93-103

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

	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (ng/mL)	8.40	1.58
Standard Deviation	0.65	0.09
% CV	7.7	5.9

Inter-Assay Precision: Two samples with different concentrations of human PCSK9 were assayed in three independent assays.

	Sample 1	Sample 2
Number of Assays	3	3
Mean Concentration (ng/mL)	12.30	2.83
Standard Deviation	1.18	0.14
% CV	9.6	4.9

Biological Samples:

Serum and Plasma

	Serum (N=22)	EDTA Plasma (N=10)	Heparin Plasma (N=10)	Citrate Plasma (N=10)
Detectable %	100	100	100	100
Mean (ng/mL)	59	44	92	82
Median (ng/mL)	57	47	85	73
Max (ng/mL)	88	68	208	178
Min (ng/mL)	23	27	52	38

Cell Culture Supernatant: Human HepG2 cells (2 x 10^5 cells/mL) were grown in Eagle's modified minimum essential media supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin sulfate. Cell culture supernatant was removed on day 4 and assayed. The level of natural human PCSK9 measured was 47 ng/mL.

Tel: 858-768-5800

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 (NaN3)	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.

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.

Tel: 858-768-5800

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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