Product Manual

Human PCSK9 ELISA Kit

Catalog Number

STA-385 **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Proprotein convertase subtilisin kexin 9 (PCSK9), also named neural apoptosis-regulated convertase 1 (NARC-1), is a member of the proteinase K subfamily of subtilisin-related serine endoproteases. PCSK9 has profound effects on plasma low-density lipoprotein (LDL)-cholesterol (LDL-C) levels through its ability to mediate LDL receptor (LDLR) protein degradation. The full-length PCSK9 protein has 692 amino acids, including a signal peptide, a prodomain, and a catalytic domain. 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 pro- domain and a 60 kDa catalytic domain. The primary physiologic function of PCSK9 is to mediate the degradation of low density lipoprotein receptor (LDLR). The link between PCSK9 and plasma LDL-C levels was first established by the discovery of missense mutations in PCSK9 that were present in patients with an autosomal dominant form of familial hypercholesterolemia (FH). Under normal physiologic conditions, the LDLR is internalized on 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 (Figure 1).

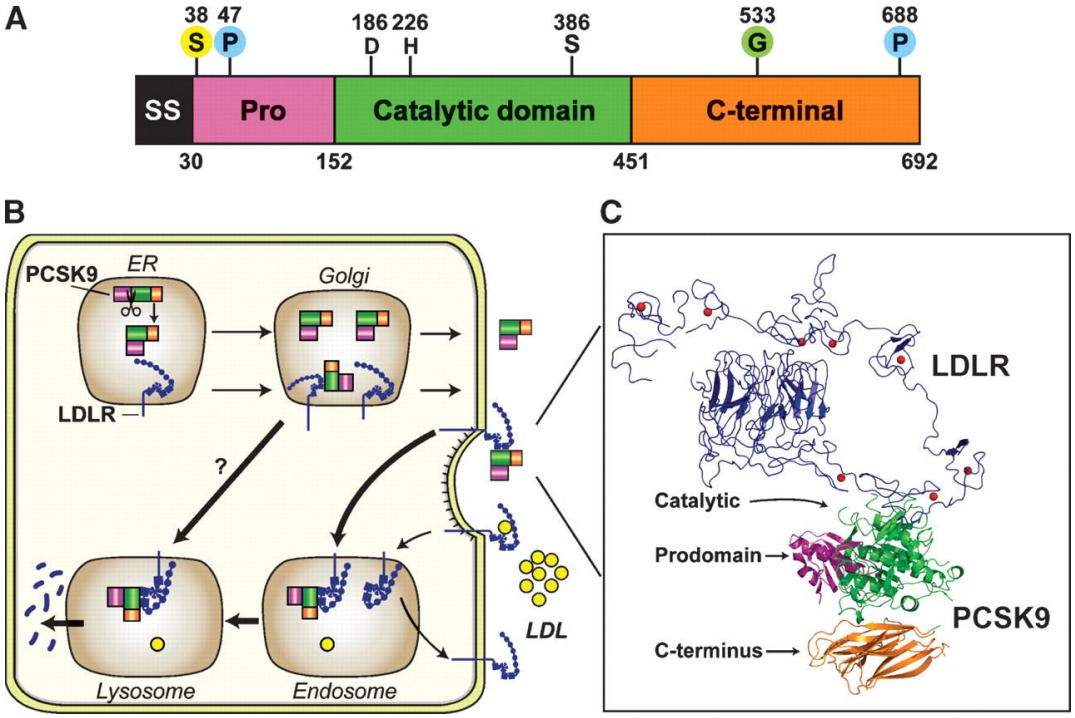

Figure 1. PCSK9-mediated degradation of the LDLR. A: Schematic of the major domains of PCSK9. B: Cellular trafficking and potential sites of PCSK9 action. C: Model for full-length LDLR bound to PCSK9.

Cell Biolabs' Human PCSK9 ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human PCSK9 in plasma, serum, cell or tissue lysate samples. The kit has a detection sensitivity limit of 150 pg/mL human PCSK9. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. STA-214: Copper (Cu++) Oxidized Human Low Density Lipoprotein (LDL)
2. STA-368: Human ApoB ELISA Kit
3. STA-369: Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
4. STA-386: Human LDLR ELISA Kit
5. STA-387: Human LOX-1 ELISA Kit
6. STA-388: Human Oxidized LDL ELISA Kit (CML-LDL Quantitation)
7. STA-389: Human Oxidized LDL ELISA Kit (HNE-LDL Quantitation)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Human PCSK9 Antibody Coated Plate (Part No. 238501): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-Human PCSK9 Antibody (500X) (Part No. 238502): One 25 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human PCSK9 Standard (Part No. 238503): One 100 µL vial of 1 µg/mL Human PCSK9 in PBS plus BSA.

Materials Not Supplied

1. Plasma, Serum, Cell or Tissue Lysate
2. PBS containing 0.1% BSA
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Human PCSK9 Standard at -20ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-Human PCSK9 Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Human PCSK9 antibody 1:500 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human PCSK9 Standard

Prepare a dilution series of human PCSK9 standards in the concentration range of 0 to 20,000 pg/mL in Assay Diluent (Table 1).

Standard Tubes	1 µg/mL Human PSCK9 Standard (µL)	Assay Diluent (µL)	Human PCSK9 (pg/mL)
1	16	784	20,000
2	400 of Tube #1	400	10,000
3	400 of Tube #2	400	5,000
4	400 of Tube #3	400	2,500
5	400 of Tube #4	400	1,250
6	400 of Tube #5	400	625
7	400 of Tube #6	400	313
8	0	400	0

Table 1. Preparation of Human PCSK9 Standards

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Plasma: Collect blood with heparin or EDTA and centrifuge for 10 minutes at 1000 g at 4ºC. Remove the plasma and assay immediately or store samples at -80ºC up to three months. Normal plasma samples require about 10 to 100 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.
- Serum: Harvest serum and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC up to three months. Normal serum samples require about 10 to 100 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA.
- Cell or Tissue Lysate: Sonicate or homogenize sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.

Assay Protocol

1. Add 100 µL of human PCSK9 unknown sample or standard to the Anti-Human PCSK9 Antibody Coated Plate. Each human PCSK9 unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at 37ºC for at least 2 hours or 4ºC overnight.
3. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Biotinylated Anti-Human PCSK9 Antibody to each well. Incubate at room temperature for 2 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above.
6. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the Human PCSK9 ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

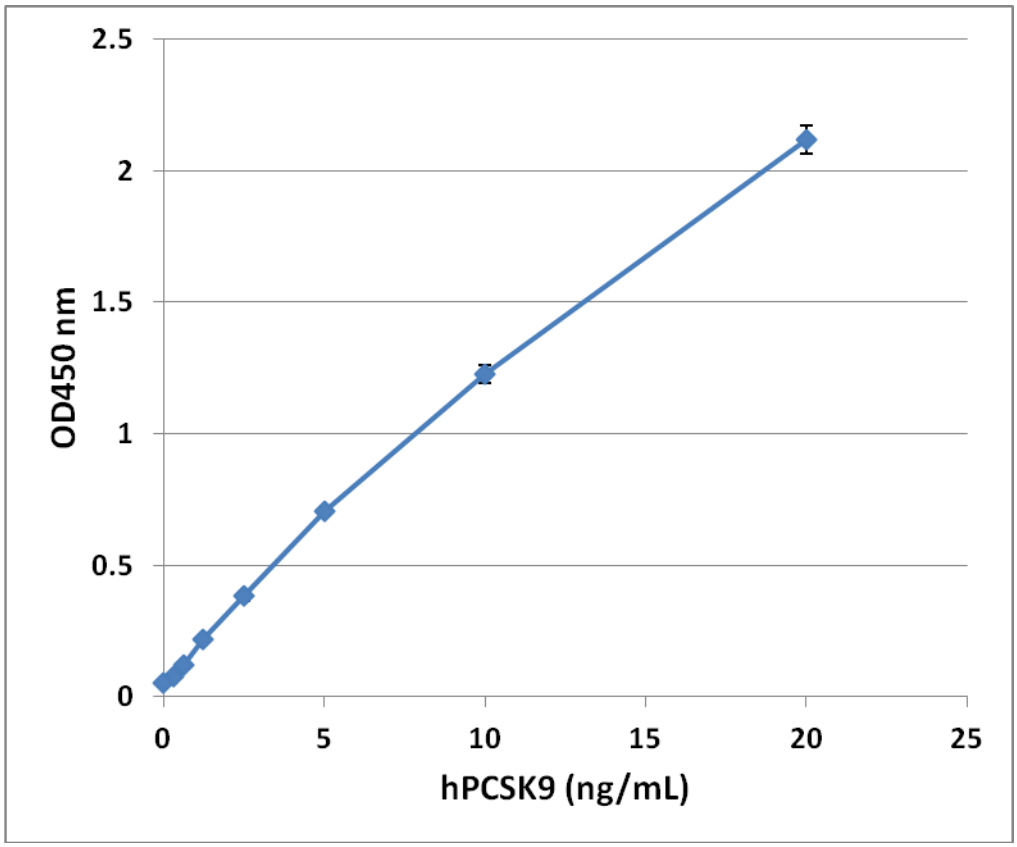

Figure 2: Human PCSK9 ELISA Standard Curve.

References

1. Abifadel, M., M. Varret, J. P. Rabes, D. Allard, K. Ouguerram, M. Devillers, C. Cruaud, S. Benjannet, L. Wickham, D. Erlich, et al. (2003) *Nat. Genet.* **34:**154–156.
2. Zaid, A., A. Roubtsova, R. Essalmani, J. Marcinkiewicz, A. Chamberland, J. Hamelin, M. Tremblay, H. Jacques, W. Jin, J. Davignon, et al. (2008) *Hepatology.* **48:** 646–654.
3. Horton, J. D., J. C. Cohen, and H. H. Hobbs. (2007) *Trends Biochem. Sci.* **32:** 71–77.
4. Seidah, N. G., S. Benjannet, L. Wickham, J. Marcinkiewicz, S. B. Jasmin, S. Stifani, A. Basak, A. Prat, and M. Chretien (2003) *Proc. Natl. Acad. Sci. USA.* **100:**928–933.

Recent Product Citations

1. Choi, H.K. et al. (2017). Welsh onion extract inhibits PCSK9 expression contributing to the maintenance of the LDLR level under lipid depletion conditions of HepG2 cells. *Food Funct*. **8**(12):4582-4591. doi: 10.1039/c7fo00562h.
2. Franzén, O. et al. (2016). Cardiometabolic risk loci share downstream cis-and trans-gene regulation across tissues and diseases. *Science.* **353**:827-830.

3. Nekaies, Y. et al. (2015). Plasma proprotein convertase subtilisin/kexin type 9 is associated with Lp (a) in type 2 diabetic patients. *J Diabetes Complicat.* doi:10.1016/j.jdiacomp.2015.08.003.
4. Jin, K. et al. (2014). Plasma PCSK9 in nephrotic syndrome and in peritoneal dialysis: a cross-sectional study. *Am J Kidney Dis*. **63**:584-589.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com