Anti-PCSK9 prodomain (Human) mAb

Cat# CY-M1032

100 µg (1 mg/mL x 100 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
KS-2C8	WB, IP	H	16-18 kDa	Mouse IgG1

Background

PCSK9 (also known as neural apoptosis-regulated convertase, NARC-1) is a 692-residue extracellular protein expressed primarily in the kidneys, liver and intestines (1) representing the 9th member of the secretory subtilase family. Various genetic observations subsequently mapped PCSK9 as the third gene (along with LDLR and APOB) to cause autosomal dominant hypercholesterolemia (ADH). These studies suggested that gain of function mutations increase plasma levels of LDL-c (2–6), whereas nonsense or missense (loss-of-function) mutations, which interfere with folding or secretion of PCSK9, lead to a reduction of plasma levels of LDL-c and an 88 % decrease in the risk of coronary heart disease (CHD) (5). In mice, adenoviral overexpression of PCSK9 results in increased plasma LDL-c level in normal mice but not in LDLR-deficient mice (7). Deletion of PCSK9 causes an increase in level of LDLR protein but not mRNA (8). These findings lead to a hypothesis that PCSK9 exerts its role in cholesterol metabolism through posttranslational down-regulation of LDLR, the receptor responsible for clearing LDL-c from plasma.

Evidence is consistent with the secreted form of PCSK9 binding directly to the LDLR and resulting in degradation of the receptor (9, 10). Zhang et al. (11) localized the binding site of PCSK9 in the LDLR to the first epidermal growth factor-like repeat (EGF-A) of the extracellular domain and showed that PCSK9 binding to this site is required for LDLR degradation. In light of these observations and the fact that PCSK9 in the circulation may cause the degradation of hepatic LDLR in the liver, PCSK9 would seem to be an attractive drug target for lowering LDLC.

Specificity/Sensitivity: Anti-PCSK9 prodomain (Human) mAb (KS-2C8) detects endogenous mature form of PCSK9 Prodomain, does not react with pro-form of PCSK9 by western blotting and immunoprecipitation.

Source/Purification: Monoclonal antibody is produced by immunizing mice with a recombinant PCSK9, corresponding full length of human PCSK9, expressed in 293 cells. IgG is purified by protein A-Sepharose chromatography.

Recommended Antibody Dilutions: Western blotting: 0.5-1 µg/mL, immunoprecipitation: 1-2 µg/sample.

Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

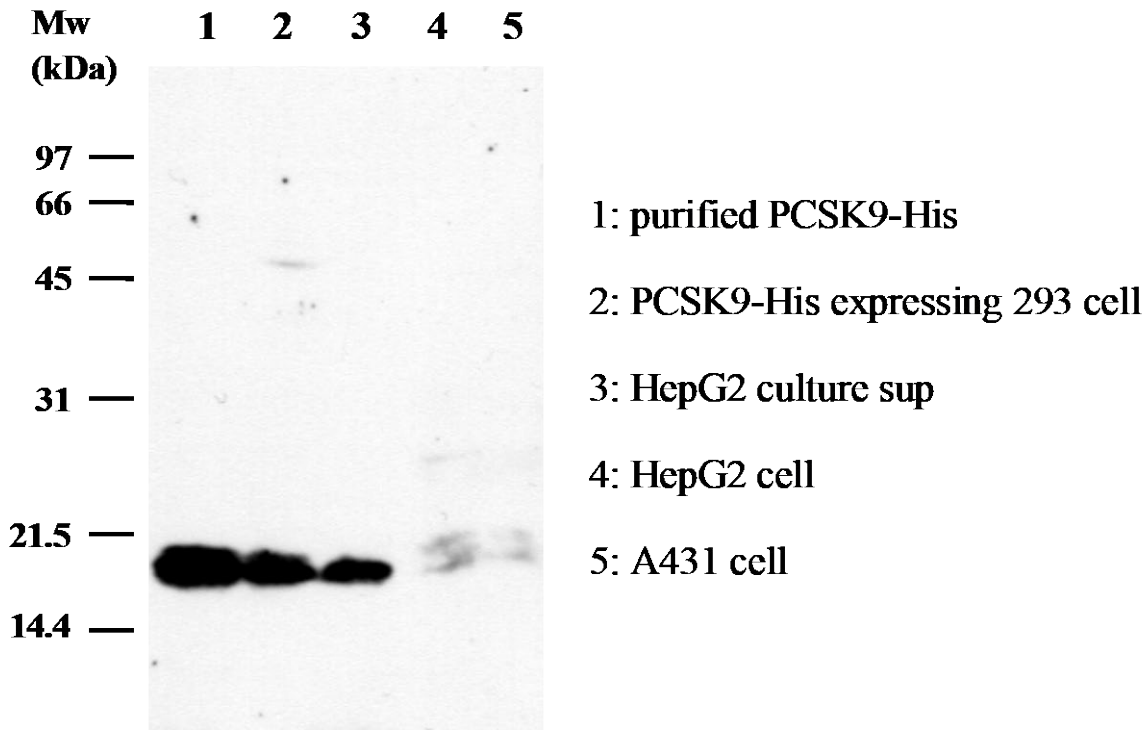
Applications Key:
WB: Western blotting, **IP:** Immunoprecipitation, **IHC:** Immunohistochemistry, **IC:** Immunocytochemistry, **F:** Flow cytometry, **E:** ELISA, **FP:** Fluorescence polarization assay

Species Cross-Reactivity Key:
H: Human, **M:** Mouse, **R:** Rat, **Hm:** Hamster, **Mk:** Monkey, **Mi:** Mink, **C:** Chicken, **X:** Xenopus, **Z:** Zebra fish (Species enclosed in parentheses are predicted to react based on 100 % sequence homology.)

References:

1. Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, Basak A, Prat A, Chretien M (2003) *Proc Natl Acad Sci USA* **100:** 928-933.
2. Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, Cruaud C, Benjannet S, Wickham L, Erlich D, et al. (2003) *Nat Genet* **34:** 154-156.
3. Leren TP (2004) *Clin Genet* **65:** 419-422.
4. Allard D, Amsellem S, Abifadel M, Trillard M, Devillers M, Luc G, Krempf M, Reznik Y, Girardet JP, Fredenrich A, et al. (2005) *Hum Mutat* **26:** 497.
5. Cohen JC, Boerwinkle E, Mosley TH, Jr, Hobbs HH (2006) *N Engl J Med* **354:** 1264-1272.
6. Berge KE, Ose L, Leren TP (2006) *Arterioscler Thromb Vasc Biol* **26:** 1094-1100.
7. Maxwell KN, Breslow JL (2004) *Proc Natl Acad Sci USA* **101:** 7100-7105.
8. Rashid S, Curtis DE, Garuti R, Anderson NN, Bashmakov Y, Ho YK, Hammer RE, Moon YA, Horton JD (2005) *Proc Natl Acad Sci USA* **102:** 5374-5379.
9. Lagace, T. A., Curtis, D. E., Garuti, R., McNutt, M. C., Park, S. W., Prather, H. B., Anderson, N. N., Ho, Y. K., Hammer, R. E., and Horton, J. D. (2006) *J. Clin. Investig*. **116:** 2995-3005
10. Cameron, J., Holla, O. L., Ranheim, T., Kulseth, M. A., Berge, K. E., and Leren, T. P. (2006) *Hum. Mol. Genet*. **15:** 1551-1558
11. Zhang, D. W., Lagace, T. A., Garuti, R., Zhao, Z., McDonald, M., Horton, J. D., Cohen, J. C., and Hobbs, H. H. (2007) *J. Biol. Chem*. **282:** 18602-18612

Fig. 1. Western blot analysis of Human PCSK9 Prodomain



Western Immunoblotting Protocol

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20 % methanol (pH 8.5)
SDS Sample Buffer (1X): 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2 % w/v SDS, 10 % glycerol, 50 mM DTT, 0.01 % w/v bromophenol blue or phenol red
Blocking Buffer: 1X TBS, 0.1 % Tween-20 with 5 % w/v nonfat dry milk; for 150 mL, add 15 mL 10X TBS to 135 mL water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 mL Tween-20 (100 %).
10X TBS (Tris-buffered saline): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer: 1X TBS, 0.1 % Tween-20 with 5 % blocking agent for 20 mL, add 2 mL 10X TBS to 18 mL water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µL Tween-20 (100 %).
Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1 % Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS, aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (100 µL per well of 6-well plate or 500 µL per plate of 10 cm^2 plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µL sample to 95–100°C for 5 minutes, cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µL onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

Membrane Blocking and Antibody Incubations

Note: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 mL TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 mL of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 mL Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 mL of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3,000 in 10 mL of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 mL of TBS/T.

Detection of Proteins

1. Incubate membrane with 4 mL ECL™ with gentle agitation for 1 minute at room temperature.

2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial ten-second exposure should indicate the proper exposure time.

Immunoprecipitation Followed by Western Immunoblotting Protocol

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Cell Lysis Buffer (1X): 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 % Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM Glycerolphosphate, 1 mM Na3VO4, 1 µg/ml Leupeptin
Note: We recommend adding 1 mM PMSF before use.
Protein A Agarose Beads: Add 5 ml of 1X PBS to 1.5 g of Protein A Agarose Beads. Shake 2 hours at 4°C; spin down. Wash pellet twice with PBS. Resuspend beads in 1 volume of PBS. (Can be stored for 2 weeks at 4°C)
3X SDS Sample Buffer: 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6 % w/v SDS, 30 % glycerol, 150 mM DTT, 0.03 % w/v bromophenol blue,
Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20 % methanol (pH 8.5)
Blocking Buffer: 1X TBS, 0.1 % Tween-20 with 5 % w/v nonfat dry milk. For 150 mL, add 15 mL 10X TBS to 135 mL water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 mL Tween-20 (100 %).
10X TBS (Tris-buffered saline): For 1 liter of 10X TBS, use 24.2 g Tris base and 80 g NaCl. Adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer: 1X TBS, 0.05 % Tween-20 with 5 % nonfat dry milk. For 20 mL, add 2 mL 10X TBS to 18 mL water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 10 µL Tween-20 (100 %).
Wash Buffer TBS/T: 1X TBS, 0.1 % Tween-20
Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1 % Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 mL 1X ice-cold Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm^2) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to microcentrifuge tubes. Keep on ice.
5. Sonicate 4 times for 5 seconds each on ice.
6. Microcentrifuge for 10 minutes at 4°C, and transfer the supernatant to a new tube. The supernatant is the cell lysate. If necessary, lysate can be stored at –80°C.

Immunoprecipitation

1. Take 200 µL cell lysate and add primary antibody; incubate with gentle rocking overnight at 4°C.
2. Add Protein A Agarose Beads (20 µL of 50 % bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
3. Microcentrifuge for 30 seconds at 4°C. Wash pellet 2 times with 500 µL of 1X Cell Lysis Buffer. Keep on ice during washes.
4. Resuspend the pellet with 20 µL 3X SDS Sample Buffer. Votex, then microcentrifuge for 30 seconds.
5. Heat the sample to 95–100°C for 2–5 minutes.
6. Load the sample (15–30 µL) on SDS-PAGE gel (12–15 %).
7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).

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