

# nicastrin siRNA (m): sc-36064

## BACKGROUND

The Presenilin 1 (PS1) and Presenilin 2 (PS2) transmembrane proteins are components of high molecular weight complexes. These complexes mediate proteolytic cleavage within the transmembrane domain of several proteins, including the  $\beta$ -Amyloid precursor protein ( $\beta$ APP) and Notch. Missense mutations in the genes encoding the Presenilin proteins increase the proteolysis of  $\beta$ APP and results in the overproduction of the neurotoxic  $\beta$ -Amyloid peptide, which results in a condition associated with Familial Alzheimer's disease (FAD). A novel component of the presenilin complex, nicastrin, is a type I transmembrane glycoprotein that is involved in mediating Notch/GLP-1 signaling. In addition, nicastrin contributes to the processing of  $\beta$ APP, which makes nicastrin an attractive potential target for modulating the production of  $\beta$ -Amyloid in patients with Alzheimer's disease. Originally purified from immunoprecipitated PS1 complexes from HEK293 cells, nicastrin contains hydrophilic amino and carboxy-terminal domains, a short, hydrophobic transmembrane domain and potential N-myristoylation and phosphorylation sites.

## REFERENCES

1. Yu, G., et al. 1998. The Presenilin 1 protein is a component of a high molecular weight intracellular complex that contains  $\beta$ -catenin. *J. Biol. Chem.* 273: 16470-16475.
2. De Strooper, B., et al. 1998. Deficiency of Presenilin 1 inhibits the normal cleavage of amyloid precursor protein. *Nature* 391: 387-390.
3. De Strooper, B., et al. 1999. A Presenilin 1-dependent  $\gamma$ -secretase-like protease mediates release of Notch intracellular domain. *Nature* 398: 518-522.
4. Song, W., et al. 1999. Proteolytic release and nuclear translocation of Notch 1 are induced by Presenilin 1 and impaired by pathogenic Presenilin 1 mutations. *Proc. Natl. Acad. Sci. USA* 96: 6959-6963.
5. Annaert, W., et al. 1999. Presenilins: molecular switches between proteolysis and signal transduction. *Trends Neurosci.* 22: 439-443.
6. Kulic, L., et al. 2000. Separation of Presenilin function in  $\beta$ -Amyloid-peptide generation and endoproteolysis of Notch. *Proc. Natl. Acad. Sci. USA* 97: 5913-5918.

## CHROMOSOMAL LOCATION

Genetic locus: Ncstn (mouse) mapping to 1 H3.

## PRODUCT

nicastrin siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see nicastrin shRNA Plasmid (m): sc-36064-SH and nicastrin shRNA (m) Lentiviral Particles: sc-36064-V as alternate gene silencing products.

For independent verification of nicastrin (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-36064A, sc-36064B and sc-36064C.

## STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNases and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

nicastrin siRNA (m) is recommended for the inhibition of nicastrin expression in mouse cells.

## SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10  $\mu$ M in 66  $\mu$ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

## GENE EXPRESSION MONITORING

nicastrin (B-3): sc-376513 is recommended as a control antibody for monitoring of nicastrin gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor nicastrin gene expression knockdown using RT-PCR Primer: nicastrin (m)-PR: sc-36064-PR (20  $\mu$ l, 458 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.