

neurolysin siRNA (h): sc-42089

BACKGROUND

Oligopeptidases are endopeptidases that act only on smaller polypeptides or oligopeptides. These enzymes are believed to influence biological functions that include the modification or destruction of peptide messenger molecules. Oligopeptidases have few naturally occurring inhibitors and possess a distinct specificity that prevents them from interacting with the ubiquitous protease inhibitor, α -2-Macroglobulin. Neuropeptidases are oligopeptidases that modify the activity of small peptide neurotransmitters and neurohormones. The neuropeptidase neurolysin is a zinc dependent metallopeptidase that acts only on short peptides and accepts a variety of cleavage-site sequences. The connecting loop regions of the five-stranded beta-sheet and the two active site helices are extended in neurolysin and may account for the ability of the enzyme to cleave a variety of sequences. Neurolysin is ubiquitously expressed within brain and specifically localizes to neuronal perikarya in rat brain.

REFERENCES

1. Barrett, A.J., et al. 1992. Oligopeptidases, and the emergence of the prolyl oligopeptidase family. *Biol. Chem. Hoppe Seyler* 373: 353-360.
2. Serizawa, A., et al. 1995. Characterization of a mitochondrial metallopeptidase reveals neurolysin as a homologue of thimet oligopeptidase. *J. Biol. Chem.* 270: 2092-2098.
3. Massarelli, E.E., et al. 1999. Differential subcellular distribution of neurolysin (EC 3.4.24.16) and thimet oligopeptidase (EC 3.4.24.15) in the rat brain. *Brain Res.* 851: 261-265.
4. Lian, W., et al. 2000. Crystallization and preliminary analysis of neurolysin. *Acta Crystallogr. D. Biol. Crystallogr.* 56:1644-1646.
5. Brown, C.K., et al. 2001. Structure of neurolysin reveals a deep channel that limits substrate access. *Proc. Natl. Acad. Sci. USA* 98: 3127-3132.

CHROMOSOMAL LOCATION

Genetic locus: NLN (human) mapping to 5q12.3.

PRODUCT

neurolysin siRNA (h) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see neurolysin shRNA Plasmid (h): sc-42089-SH and neurolysin shRNA (h) Lentiviral Particles: sc-42089-V as alternate gene silencing products.

For independent verification of neurolysin (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-42089A, sc-42089B and sc-42089C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

APPLICATIONS

neurolysin siRNA (h) is recommended for the inhibition of neurolysin expression in human 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 μ M in 66 μ 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

neurolysin (B-3): sc-398420 is recommended as a control antibody for monitoring of neurolysin 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 neurolysin gene expression knockdown using RT-PCR Primer: neurolysin (h)-PR: sc-42089-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.