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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

CNPase siRNA (h): sc-44377

BACKGROUND

2',3'-cyclic nucleotide-3'-phosphodiesterase (CNPase) is a membrane-bound enzyme that can link tubulin to membranes and may regulate cytoplasmic microtubule distribution. CNPase acts as a microtubule-associated protein by promoting microtubule assembly; this activity resides in the C-terminus of the enzyme. CNPase is firmly associated with tubulin from brain tissue and thyroid cells and can be found at high concentrations in central nervous system myelin and in the outer segments of photoreceptors in the retina. Acute lead intoxication leads to disturbances in CNPase activity and the morphology of myelin.

REFERENCES

1. Sprinkle, T.J., et al. 1987. Monoclonal antibody production to human and bovine 2',3'-cyclic nucleotide-3'-phosphodiesterase (CNPase): high-specificity recognition in whole brain acetone powders and conservation of sequence between CNP1 and CNP2. *Brain Res.* 426: 349-357.
2. Vogel, U., et al. 1988. Molecular structure, localization and possible functions of the myelin-associated enzyme 2',3'-cyclic nucleotide-3'-phosphodiesterase. *J. Neurochem.* 50: 1667-1677.
3. Dabrowska-Bouta, B., et al. 2000. Acute lead intoxication *in vivo* affects myelin membrane morphology and CNPase activity. *Exp. Toxicol. Pathol.* 52: 257-263.
4. Bifulco, M., et al. 2002. 2',3'-cyclic nucleotide-3'-phosphodiesterase: a membrane-bound, microtubule-associated protein and membrane anchor for tubulin. *Proc. Natl. Acad. Sci. USA* 99: 1807-1812.
5. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 123830. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: CNP (human) mapping to 17q21.2.

PRODUCT

CNPase 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 CNPase shRNA Plasmid (h): sc-44377-SH and CNPase shRNA (h) Lentiviral Particles: sc-44377-V as alternate gene silencing products.

For independent verification of CNPase (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-44377A, sc-44377B and sc-44377C.

RESEARCH USE

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

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

CNPase siRNA (h) is recommended for the inhibition of CNPase 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

CNPase (H-2): sc-166558 is recommended as a control antibody for monitoring of CNPase 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 CNPase gene expression knockdown using RT-PCR Primer: CNPase (h)-PR: sc-44377-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.