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SANTA CRUZ BIOTECHNOLOGY, INC.

NTR3 siRNA (m): sc-42120

BACKGROUND

Neurotensin (NT) initiates an intracellular response by interacting with the G protein-coupled receptors NTR1 (NTS1 receptor, high affinity NTR) and NTR2 (NTS2 receptor, levocabastine-sensitive Neurotensin receptor), and the type I receptor NTR3 (NTS3 receptor, sortilin-1, Gp95). NT has a wide distribution in regions of the brain and in peripheral tissues where NT receptors can contribute to hypotension, hyperglycemia, hypothermia, antinociception and regulation of intestinal motility and secretion. HL-60 cells express NTR1, which can couple to G_q, $G_{i/o}$ or G_s. Alternative splicing of rat NTR2 can generate a 5-transmembrane domain variant isoform that is coexpressed with the full-length NTR2 throughout the brain and spinal cord. NTR3 activation in the murine microglial cell line N11 induces MIP-2, MCP-1, IL-1β and TNFα in an ERK 1/2 and Akt kinase-dependent manner.

REFERENCES

1. Nielsen, M.S., et al. 1999. Sortilin/NTR3 binds and mediates degradation of lipoprotein lipase. J. Biol. Chem. 274: 8832-8836.
2. Choi, S.Y., et al. 1999. Characterization of high affinity Neurotensin receptor NTR1 in HL-60 cells and its downregulation during granulocytic differentiation. Br. J. Pharmacol. 126: 1050-1056.
3. Navarro, V., et al. 2002. Shedding of the luminal domain of the NTR3/sortilin in the HT29 cell line. Biochem. Biophys. Res. Commun. 298: 760-764.
4. Martin, S., et al. 2003. Involvement of the NTR3 in the Neurotensin-induced migration of human microglia. J. Neurosci. 23: 1198-1205.
5. Dicou, E., et al. 2004. NTR3/sortilin mediates Neurotensin-induced cytokine/chemokine expression in a murine microglial cell line. J. Neurosci. Res. 78: 92-99.
6. Leonetti, M., et al. 2004. Specific involvement of Neurotensin type 1 receptor in the Neurotensin-mediated *in vivo* Dopamine efflux using knock-out mice. J. Neurochem. 89: 1-6.
7. Chen, L., et al. 2004. Neurotensin depolarizes globus pallidus neurons in rats via Neurotensin type-1 receptor. Neuroscience 125: 853-859.

CHROMOSOMAL LOCATION

Genetic locus: Sort1 (mouse) mapping to 3 F3.

PRODUCT

NTR3 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NTR3 shRNA Plasmid (m): sc-42120-SH and NTR3 shRNA (m) Lentiviral Particles: sc-42120-V as alternate gene silencing products.

For independent verification of NTR3 (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-42120A, sc-42120B and sc-42120C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NTR3 siRNA (m) is recommended for the inhibition of NTR3 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 µM in 66 µl. 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

NTR3 (G-11): sc-376561 is recommended as a control antibody for monitoring of NTR3 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 NTR3 gene expression knockdown using RT-PCR Primer: NTR3 (m)-PR: sc-42120-PR (20 µl). 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.

PROTOCOLS

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