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Orexin-A/B siRNA (m): sc-42153

BACKGROUND

The hypothalamus is essential for maintaining homeostasis by integrating the vertebrate endocrine and nervous systems, thereby controlling temperature, thirst and hunger. Orexin-A and Orexin-B (also designated hypocretins) are hypothalamic neuropeptides that are derived from a single precursor, prepro-orexin, by proteolytic processing. These peptides bind to and activate two closely related, G protein-coupled receptors, designated Orexin receptor-1 (Orexin R-1) and Orexin receptor-2 (Orexin R-2). Orexin-A protein and prepro-orexin mRNA are localized to neurons within the lateral section of the hypothalamus, designated the "feeding center". Prepro-orexin mRNA is upregulated during fasting, suggesting that orexins may play a role in the central feedback mechanism that regulates feeding behavior. Orexin has been shown to increase the release of GABA and glutamate from axons, a response seen as a result of most synaptic activities in the hypothalamic region.

REFERENCES

1. Campbell, N. 1990. Biology, 2nd edition Redwood City: The Benjamin/Cummings Publishing Company, Inc. 916-921.
2. Sakurai, T., et al. 1998. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92: 573-585.
3. de Lecea, L., et al. 1998. The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc. Natl. Acad. Sci. USA* 95: 322-327.
4. Wolf, G. 1998. Orexins: a newly discovered family of hypothalamic regulators of food intake. *Nutr. Rev.* 56: 172-173.
5. van der Pol, et al. 1998. Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. *J. Neurosci.* 18: 7962-7971.

CHROMOSOMAL LOCATION

Genetic locus: Hcrt (mouse) mapping to 11 D.

PRODUCT

Orexin-A/B 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Orexin-A/B shRNA Plasmid (m): sc-42153-SH and Orexin-A/B shRNA (m) Lentiviral Particles: sc-42153-V as alternate gene silencing products.

For independent verification of Orexin-A/B (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-42153A, sc-42153B and sc-42153C.

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

Orexin-A/B siRNA (m) is recommended for the inhibition of Orexin-A/B 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 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

Orexin-A (KK09): sc-80263 is recommended as a control antibody for monitoring of Orexin-A/B 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 Orexin-A/B gene expression knockdown using RT-PCR Primer: Orexin-A/B (m)-PR: sc-42153-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.