SANTA CRUZ BIOTECHNOLOGY, INC.

Orexin R-1 siRNA (h): sc-36131

BACKGROUND

The hypothalamus is essential for maintaining homeostasis by integrating the vertebrate endocrine and nervous systems, thereby controlling temperature, thrist 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. 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.
2. Lund, P.E., et al. 2000. The Orexin OX1 receptor activates a novel Ca^{2+} influx pathway necessary for coupling to phospholipase C. J. Biol. Chem. 275: 30806-30812.
3. Hervieu, G.J., et al. 2001. Gene expression and protein distribution of the Orexin-1 receptor in the rat brain and spinal cord. Neuroscience 103: 777-797.
4. Guan, J.L., et al. 2002. Ultrastructural localization of Orexin-1 receptor in pre and post-synaptic neurons in the rat arcuate nucleus. Neurosci. Lett. 329: 209-212.
5. Hilairet, S., et al. 2003. Hypersensitization of the Orexin-1 receptor by the CB1 receptor: evidence for cross-talk blocked by the specific CB1 antagonist, SR141716. J. Biol. Chem. 278: 23731-23737.
6. Barreiro, M.L., et al. 2004. Orexin-1 receptor messenger ribonucleic acid expression and stimulation of testosterone secretion by Orexin-A in rat testis. Endocrinology 145: 2297-2306.

CHROMOSOMAL LOCATION

Genetic locus: HCRTR1 (human) mapping to 1p35.2.

PRODUCT

Orexin R-1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Orexin R-1 shRNA Plasmid (h): sc-36131-SH and Orexin R-1 shRNA (h) Lentiviral Particles: sc-36131-V as alternate gene silencing products.

For independent verification of Orexin R-1 (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-36131A, sc-36131B and sc-36131C.

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

Orexin R-1 siRNA (h) is recommended for the inhibition of Orexin R-1 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 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

Orexin R-1/2 (E-9): sc-166111 is recommended as a control antibody for monitoring of Orexin R-1 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 Orexin R-1 gene expression knockdown using RT-PCR Primer: Orexin R-1 (h)-PR: sc-36131-PR (20 µl, 588 bp). 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.