

pro-MCH siRNA (h): sc-42015

BACKGROUND

Melanin-concentrating hormone (MCH) is a 19 amino acid cyclic neuropeptide derived from a 165 amino acid pro-MCH precursor. In addition to the hormone, the pro-MCH precursor contains a 144 amino acid mature MCH as well as a 12 amino acid neuropeptide glycine-glutamic acid (NGE) and a 19 amino acid neuropeptide glutamic acid-isoleucine (NEI). Mainly expressed in the hypothalamus, MCH modulates feeding behavior, aggression, anxiety, arousal and reproductive function in mammals by controlling the release of luteinizing hormone (LH). The melanin-concentrating hormone receptor (MCHR) also designated SLC-1 is a glycosylated G protein-coupled receptor. MCHR mediates the effects of MCH through $G_{\alpha i}$ and/or $G_{\alpha q}$ signaling and is expressed in several regions of the brain, including the cerebral cortex, amygdala, thalamus and hypothalamus. MCH and MCHR have also been implicated in stimulating leptin expression and secretion in adipocytes, suggesting that the melanin-concentrating hormone and its receptor may be potential targets for modulating obesity.

REFERENCES

1. Drozd, R., et al. 1999. (D-(p-benzoylphenylalanine) 13, tyrosine19)-melanin-concentrating hormone, a potent analogue for MCH receptor crosslinking. *J. Pept. Sci.* 5: 234-242.
2. Saito, Y., et al. 1999. Molecular characterization of the melanin-concentrating-hormone receptor. *Nature* 400: 265-269.
3. Murray, J.F., et al. 2000. The influence of gonadal steroids on pre-pro melanin-concentrating hormone mRNA in female rats. *J. Neuroendocrinol.* 12: 53-59.
4. Murray, J.F., et al. 2000. Melanin-concentrating hormone, melanocortin receptors and regulation of luteinizing hormone release. *J. Neuroendocrinol.* 12: 217-223.
5. Hawes, B.E., et al. 2000. The melanin-concentrating hormone receptor couples to multiple G proteins to activate diverse intracellular signaling pathways. *Endocrinology* 141: 4524-4532.
6. Bradley, R.L., et al. 2000. Melanin-concentrating hormone regulates leptin synthesis and secretion in rat adipocytes. *Diabetes* 49: 1073-1077.

CHROMOSOMAL LOCATION

Genetic locus: PMCH (human) mapping to 12q23.2.

PRODUCT

pro-MCH 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 pro-MCH shRNA Plasmid (h): sc-42015-SH and pro-MCH shRNA (h) Lentiviral Particles: sc-42015-V as alternate gene silencing products.

For independent verification of pro-MCH (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-42015A, sc-42015B and sc-42015C.

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

pro-MCH siRNA (h) is recommended for the inhibition of pro-MCH 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

pro-MCH (1D1): sc-293231 is recommended as a control antibody for monitoring of pro-MCH 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 pro-MCH gene expression knockdown using RT-PCR Primer: pro-MCH (h)-PR: sc-42015-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.

PROTOCOLS

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