

Ob-R siRNA (r): sc-270697

BACKGROUND

Although there is substantial evidence that body weight is physiologically regulated, the molecular basis of obesity is unknown. Five single-gene mutations in mice that result in an obese phenotype have been identified. The first such recessive obesity mutation, the obese mutation (Ob), was identified in 1950. Mutation of Ob results in profound obesity and type II diabetes as part of a syndrome that resembles morbid obesity in humans. It has been postulated that the Ob gene product may function as a component of a signaling pathway in adipose tissue that functions to regulate body fat depot size. The cloning and sequence analysis of the mouse Ob gene and its human homolog has recently been described. Ob encodes an adipose tissue-specific mRNA with a highly conserved 167 amino acid open reading frame. The predicted amino acid sequence is 84% identical between human and mouse and has the features of a secreted protein. A nonsense mutation in codon 105 has been found in the original congenic C57BL/6J Ob/Ob mouse strain. The Ob gene encodes the protein leptin. The leptin receptor, designated Ob-R, has been shown to be a single membrane-spanning receptor that most resembles the gp130 signal transducing component of the IL-6, G-CSF and LIF receptor. Ob-R mRNA is expressed in the choroid plexus and hypothalamus.

REFERENCES

1. Ingalls, A.M., et al. 1950. Obese, a new mutation in the house mouse. *J. Hered.* 41: 317-318.
2. Friedman, J.M., et al. 1991. Molecular mapping of the mouse Ob mutation. *Genomics* 11: 1054-1062.
3. Friedman, J.M. and Leibel, R.L. 1992. Tackling a weighty problem. *Cell* 69: 217-220.
4. Rink, T.J. 1994. In search of a satiety factor. *Nature* 372: 406-407.
5. Zhang, Y., et al. 1994. Positional cloning of the mouse obese gene and its human homologue. *Nature* 372: 425-431.
6. Tartaglia, L.A., et al. 1995. Identification and expression cloning of a leptin receptor, Ob-R. *Cell* 83: 1263-1271.
7. Barinaga, M. 1996. Researchers nail down leptin receptor. *Science* 271: 913.

CHROMOSOMAL LOCATION

Genetic locus: *Lepr* (rat) mapping to 5q33.

PRODUCT

Ob-R siRNA (r) 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 Ob-R shRNA Plasmid (r): sc-270697-SH and Ob-R shRNA (r) Lentiviral Particles: sc-270697-V as alternate gene silencing products.

For independent verification of Ob-R (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270697A, sc-270697B and sc-270697C.

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

Ob-R siRNA (r) is recommended for the inhibition of Ob-R expression in rat 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

Ob-R (B-3): sc-8391 is recommended as a control antibody for monitoring of Ob-R 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 Ob-R gene expression knockdown using RT-PCR Primer: Ob-R (r)-PR: sc-270697-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.