

OPG siRNA (m): sc-40153

BACKGROUND

Bone morphogenesis and remodeling involve the formation of bone from osteoblasts and the resorption of bone by osteoclasts. The cytokine osteoprotegerin (OPG), also designated osteoclastogenesis inhibitory factor (OCIF), is known to inhibit osteoclast formation. A secreted glycoprotein, OPG is a member of the TNF receptor family that increases bone density and volume. OPG is thought to inhibit osteoclastogenesis by disrupting the cell-to-cell signaling between osteoblastic stromal cells and osteoclast progenitors. OPG is known to bind to TRAIL, a death domain-containing protein, and to inhibit TRAIL apoptosis in Jurkat cells. OPG also binds to osteoclast differentiation factor (ODF), also known as TRANCE/RANKL, a membrane-bound protein belonging to the TNF ligand family. Both TNF α and TNF β upregulate OPG expression, while the bone resorbing agent prostaglandin E₂ downregulates OPG.

REFERENCES

1. Simonet, W.S., et al. 1997. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 89: 309-319.
2. Hill, P.A. 1998. Bone remodeling. *Br. J. Orthod.* 25: 101-107.
3. Yasuda, H., et al. 1998. Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis *in vitro*. *Endocrinology* 139: 1329-1337.
4. Yasuda, H., et al. 1998. Osteoclast differentiation is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc. Natl. Acad. Sci. USA* 95: 3597-3602.
5. Brandstrom, H., et al. 1998. Regulation of osteoprotegerin mRNA levels by prostaglandin E₂ in human bone marrow stroma cells. *Biochem. Biophys. Res. Commun.* 247: 338-341.
6. Brandstrom, H., et al. 1998. Tumor necrosis factor- α and - β upregulate the levels of osteoprotegerin mRNA in human osteosarcoma MG-63 cells. *Biochem. Biophys. Res. Commun.* 248: 454-457.
7. Emery, J.G., et al. 1998. Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. *J. Biol. Chem.* 273: 14363-14367.

CHROMOSOMAL LOCATION

Genetic locus: Tnfrsf11b (mouse) mapping to 15 D1.

PRODUCT

OPG 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 OPG shRNA Plasmid (m): sc-40153-SH and OPG shRNA (m) Lentiviral Particles: sc-40153-V as alternate gene silencing products.

For independent verification of OPG (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-40153A, sc-40153B and sc-40153C.

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

OPG siRNA (m) is recommended for the inhibition of OPG 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

OPG (E-10): sc-390518 is recommended as a control antibody for monitoring of OPG 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 OPG gene expression knockdown using RT-PCR Primer: OPG (m)-PR: sc-40153-PR (20 μ l, 434 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Diarra, D., et al. 2007. Dickkopf-1 is a master regulator of joint remodeling. *Nat. Med.* 13: 156-163.

RESEARCH USE

For research use only, not for use in diagnostic procedures.