SANTA CRUZ BIOTECHNOLOGY, INC.

Sclerostin siRNA (h): sc-61503

BACKGROUND

Sclerosteosis (SOST), an autosomal recessive sclerosing bone dysplasia, and Van Buchem disease, a closely related disorder, cause abnormal, progressive bone overgrowth. SOST is associated with mutations in the SOST gene and leads to gigantism, entrapment of the seventh and eighth cranial nerves and possibly also distortion of the facies. Van Buchem disease is associated with a 52 kb deletion downstream of the SOST gene that probably affects transcription of the gene. Sclerostin, the protein encoded by the SOST gene, is important for bone homeostasis. It is a secreted protein that inhibits bone formation. Sclerostin is generally expressed at low levels, but high expression of Sclerostin can be detected in bone, cartilage, liver, bone marrow and kidney tissue.

REFERENCES

1. Kusu, N., et al. 2003. Sclerostin is a novel secreted osteoclast-derived bone morphogenetic protein antagonist with unique ligand specificity. J. Biol. Chem. 278: 24113-24117.
2. Sutherland, M.K., et al. 2004. Sclerostin promotes the apoptosis of human osteoblastic cells: a novel regulation of bone formation. Bone 35: 828-835.
3. Winkler, D.G., et al. 2005. Sclerostin inhibition of Wnt-3a-induced C3H10T1/2 cell differentiation is indirect and mediated by bone morphogenetic proteins. J. Biol. Chem. 280: 2498-2502.
4. Li, X., et al. 2005. Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. J. Biol. Chem. 280: 19883-19887.
5. van Bezooijen, R.L., et al. 2005. SOST/Sclerostin, an osteocyte-derived negative regulator of bone formation. Cytokine Growth Factor Rev. 16: 319-327.
6. van Bezooijen, R.L., et al. 2005. Bone morphogenetic proteins and their antagonists: the sclerostin paradigm. J. Endocrinol. Invest. 28: 15-17.
7. Poole, K.E., et al. 2005. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. FASEB J. 19: 1842-1844.

CHROMOSOMAL LOCATION

Genetic locus: SOST (human) mapping to 17q21.31.

PRODUCT

Sclerostin 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 Sclerostin shRNA Plasmid (h): sc-61503-SH and Sclerostin shRNA (h) Lentiviral Particles: sc-61503-V as alternate gene silencing products.

For independent verification of Sclerostin (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-61503A, sc-61503B and sc-61503C.

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 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

Sclerostin siRNA (h) is recommended for the inhibition of Sclerostin 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

Sclerostin (C-11): sc-518161 is recommended as a control antibody for monitoring of Sclerostin 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 Sclerostin gene expression knockdown using RT-PCR Primer: Sclerostin (h)-PR: sc-61503-PR (20 µl). 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.