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OPN siRNA (r): sc-270052

BACKGROUND

Osteopontin (OPN), also designated bone sialoprotein 1, urinary stone protein, SPP-1, ETA-1, nephropontin and uropontin, is an extracellular matrix cell adhesion phosphoglycoprotein. OPN is deposited into unmineralized matrix prior to calcification leading to localization at various tissue interfaces including cement lines, lamina limitans and between collagen fibrils of fully matured hard tissues. While OPN is a major product of osteoblasts, it is also synthesized by brain and kidney cells. OPN isolated from or secreted by various tissues ranges in molecular weight due to posttranslational modifications. OPN functions as a substrate for transglutaminase and is involved in cell adhesion, chemoattraction and immunomodulation.

REFERENCES

- Butler, W.T. 1989. The nature and significance of osteopontin. *Connect. Tissue Res.* 23: 123-136.
- Singh, R.P., et al. 1990. Definition of a specific interaction between the early T lymphocyte activation 1 (ETA-1) protein and murine macrophages *in vitro* and its effect upon macrophages *in vivo*. *J. Exp. Med.* 171: 1931-1942.
- Prince, C.W., et al. 1991. Osteopontin, a substrate for transglutaminase and Factor XIII activity. *Biochem. Biophys. Res. Commun.* 177: 1205-1210.
- Denhardt, D.T., et al. 1993. Osteopontin: a protein with diverse functions. *FASEB J.* 7: 1475-1482.
- Butler, W.T. 1995. Structural and functional domains of osteopontin. *Ann. N.Y. Acad. Sci.* 760: 6-11.
- Weber, G.F., et al. 1996. The immunology of ETA-1/osteopontin. *Cytokine Growth Factor Rev.* 7: 241-248.

CHROMOSOMAL LOCATION

Genetic locus: Spp1 (rat) mapping to 14p21.

PRODUCT

OPN 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 OPN shRNA Plasmid (r): sc-270052-SH and OPN shRNA (r) Lentiviral Particles: sc-270052-V as alternate gene silencing products.

For independent verification of OPN (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-270052A, sc-270052B and sc-270052C.

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

OPN siRNA (r) is recommended for the inhibition of OPN 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

OPN (AKm2A1): sc-21742 is recommended as a control antibody for monitoring of OPN 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 OPN gene expression knockdown using RT-PCR Primer: OPN (r)-PR: sc-270052-PR (20 μ l, 510 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Jin, Y.C., et al. 2016. Intranasal delivery of RGD motif-containing osteopontin icosamer confers neuroprotection in the postischemic brain via $\alpha_v\beta_3$ integrin binding. *Mol. Neurobiol.* 53: 5652-5663.

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.