

SPARC siRNA (m): sc-41034

BACKGROUND

SPARC (for secreted protein acidic and rich in cysteine) is a phosphorylated, acidic, glycine-rich glycoprotein that is secreted by endothelial cells and is present in large amounts in the parietal endoderm of mouse embryos and in human placenta. It is identical to osteonectin, a protein important to bone calcification that is highly conserved between species. SPARC, which can be selectively expressed by the endothelium in response to certain types of injury, induces rounding in adherent endothelial cells *in vitro*. It regulates endothelial barrier function through F-Actin-dependent changes in cell shape, coincident with the appearance of intercellular gaps, which provide a paracellular pathway for extravasation of macromolecules.

REFERENCES

1. Termine, J.D., et al. 1981. Osteonectin, a bone-specific protein linking mineral to collagen. *Cell* 26: 99-105.
2. Findlay, D.M., et al. 1988. Isolation of the osteonectin gene: evidence that a variable region of the osteonectin molecule is encoded within one exon. *Biochemistry* 27: 1483-1489.
3. Naylor, S.L., et al. 1989. The human osteonectin gene on chromosome 5 is polymorphic. *Cytogenet. Cell Genet.* 51: 1051.
4. Le Beau, M.M., et al. 1993. Cytogenetic and molecular delineation of the smallest commonly deleted region of chromosome 5 in malignant myeloid diseases. *Proc. Natl. Acad. Sci. USA* 90: 5484-5488.
5. Goldblum, S.E., et al. 1994. SPARC (secreted protein acidic and rich in cysteine) regulates endothelial cell shape and barrier function. *Proc. Natl. Acad. Sci. USA* 91: 3448-3452.

CHROMOSOMAL LOCATION

Genetic locus: Sparc (mouse) mapping to 11 B1.3.

PRODUCT

SPARC 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 SPARC shRNA Plasmid (m): sc-41034-SH and SPARC shRNA (m) Lentiviral Particles: sc-41034-V as alternate gene silencing products.

For independent verification of SPARC (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-41034A, sc-41034B and sc-41034C.

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.

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

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

SPARC (AON-1): sc-33645 is recommended as a control antibody for monitoring of SPARC 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 SPARC gene expression knockdown using RT-PCR Primer: SPARC (m)-PR: sc-41034-PR (20 μ l, 514 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.