

PHOSPHO1 siRNA (h): sc-93674

BACKGROUND

PHOSPHO1 (phosphatase, orphan 1), also referred to as phosphoethanolamine/phosphocholine phosphatase, is a 267 amino acid phosphatase that is a member of the haloacid dehalogenase (HAD) superfamily of magnesium-dependent hydrolases. PHOSPHO1 is highly expressed in bone and cartilage and localizes to the osteoid layer of the periosteum. PHOSPHO1 is restricted to sites of mineralization and its inhibition decreases the ability of matrix vesicles to calcify in bone, suggesting that the protein may play a role in the matrix mineralization process during skeletal development. PHOSPHO1 cleaves phosphoethanolamine and phosphocholine to generate inorganic phosphate for bone mineralization. PHOSPHO1 contains three catalytic motifs that are conserved within the haloacid dehalogenase superfamily.

REFERENCES

1. Houston, B., et al. 2002. Chromosomal localization of the chicken and mammalian orthologues of the orphan phosphatase PHOSPHO1 gene. *Anim. Genet.* 33: 451-454.
2. Stewart, A.J., et al. 2003. Comparative modelling of human PHOSPHO1 reveals a new group of phosphatases within the haloacid dehalogenase superfamily. *Protein Eng.* 16: 889-895.
3. Roberts, S.J., et al. 2004. Human PHOSPHO1 exhibits high specific phosphoethanolamine and phosphocholine phosphatase activities. *Biochem. J.* 382: 59-65.
4. Houston, B., et al. 2004. PHOSPHO1-A novel phosphatase specifically expressed at sites of mineralisation in bone and cartilage. *Bone* 34: 629-637.
5. Roberts, S.J., et al. 2005. Probing the substrate specificities of human PHOSPHO1 and PHOSPHO2. *Biochim. Biophys. Acta* 1752: 73-82.
6. Stewart, A.J., et al. 2006. The presence of PHOSPHO1 in matrix vesicles and its developmental expression prior to skeletal mineralization. *Bone* 39: 1000-1007.
7. Roberts, S., et al. 2007. Functional involvement of PHOSPHO1 in matrix vesicle-mediated skeletal mineralization. *J. Bone Miner. Res.* 22: 617-627.

CHROMOSOMAL LOCATION

Genetic locus: PHOSPHO1 (human) mapping to 17q21.32.

PRODUCT

PHOSPHO1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PHOSPHO1 shRNA Plasmid (h): sc-93674-SH and PHOSPHO1 shRNA (h) Lentiviral Particles: sc-93674-V as alternate gene silencing products.

For independent verification of PHOSPHO1 (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-93674A, sc-93674B and sc-93674C.

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

PHOSPHO1 siRNA (h) is recommended for the inhibition of PHOSPHO1 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 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

PHOSPHO1 (IL-91): sc-100351 is recommended as a control antibody for monitoring of PHOSPHO1 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 PHOSPHO1 gene expression knockdown using RT-PCR Primer: PHOSPHO1 (h)-PR: sc-93674-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.