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Type I 4-phosphatase siRNA (h): sc-44177

BACKGROUND

The inositol polyphosphate 4-phosphatases selectively remove the phosphate from the 4-position of various phosphatidylinositols, which generates second messengers in response to extracellular signals. Both the Type I and Type II 4-phosphatases have a molecular mass of approximately 105 kDa and catalyze the hydrolysis of phosphatidylinositol 3,4-bisphosphate, inositol 1,3,4-trisphosphate and inositol 3,4-bisphosphate. Type I and Type II 4-phosphatases are both alternatively spliced into two isoforms, α and β , which have been detected in human platelets, rat brain, heart, skeletal muscle and spleen; and all isoforms contain a conserved motif CKSAKDRT, which contains the active site consensus sequence C-X5-R. Both Type I and II 4-phosphatases are thought to regulate the level of intracellular calcium by acting as signal terminating enzymes.

REFERENCES

1. Bansal, V.S., et al. 1990. The isolation and characterization of inositol polyphosphate 4-phosphatase. *J. Biol. Chem.* 265: 1806-1811.
2. Norris, F.A., et al. 1995. The isolation and characterization of cDNA encoding human and rat brain inositol polyphosphate 4-phosphatase. *J. Biol. Chem.* 270: 16128-16133.
3. Norris, F.A., et al. 1997. Inositol polyphosphate 4-phosphatase is inactivated by calpain-mediated proteolysis in stimulated human platelets. *J. Biol. Chem.* 272: 10987-10989.
4. Norris, F.A., et al. 1997. The cDNA cloning and characterization of inositol polyphosphate 4-phosphatase Type II. Evidence for conserved alternative splicing in the 4-phosphatase family. *J. Biol. Chem.* 272: 23859-23864.
5. Majerus, P.W., et al. 1999. The role of phosphatases in inositol signaling reactions. *J. Biol. Chem.* 274: 10669-10672.

CHROMOSOMAL LOCATION

Genetic locus: INPP4A (human) mapping to 2q11.2.

PRODUCT

Type I 4-phosphatase 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 Type I 4-phosphatase shRNA Plasmid (h): sc-44177-SH and Type I 4-phosphatase shRNA (h) Lentiviral Particles: sc-44177-V as alternate gene silencing products.

For independent verification of Type I 4-phosphatase (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-44177A, sc-44177B and sc-44177C.

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

Type I 4-phosphatase siRNA (h) is recommended for the inhibition of Type I 4-phosphatase 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

Type I 4-phosphatase (E-2): sc-390549 is recommended as a control antibody for monitoring of Type I 4-phosphatase 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 Type I 4-phosphatase gene expression knockdown using RT-PCR Primer: Type I 4-phosphatase (h)-PR: sc-44177-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.