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# Type II 4-phosphatase shRNA (h) Lentiviral Particles: sc-44178-V

## 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 an approximate molecular mass of 106 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,  $\alpha$  and  $\beta$ , 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. Majerus, P.W., et al. 1999. The role of phosphatases in inositol signaling reactions. *J. Biol. Chem.* 274: 10669-10672.

## CHROMOSOMAL LOCATION

Genetic locus: INPP4B (human) mapping to 4q31.21.

## PRODUCT

Type II 4-phosphatase shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Type II 4-phosphatase siRNA (h): sc-44178 and Type II 4-phosphatase shRNA Plasmid (h): sc-44178-SH as alternate gene silencing products.

## APPLICATIONS

Type II 4-phosphatase shRNA (h) Lentiviral Particles is recommended for the inhibition of Type II 4-phosphatase expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

Type II 4-phosphatase (N-20): sc-12318 is recommended as a control antibody for monitoring of Type II 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Type II 4-phosphatase gene expression knockdown using RT-PCR Primer: Type II 4-phosphatase (h)-PR: sc-44178-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

## PROTOCOLS

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