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# PDZ-RhoGEF shRNA (h) Lentiviral Particles: sc-45823-V

## BACKGROUND

The multidomain (RGS)-containing RhoGEFs represent a family of guanine nucleotide exchange factors that stabilize the nucleotide-free state of small GTPases through their DH/PH domains, leading to the exchange of GDP to GTP. Uniquely, PDZ-RhoGEF, also known as Rho guanine nucleotide exchange factor 11 and ARHGEF11, binds tightly to both nucleotide-free and activated Rho A, therefore playing a role as a primary regulator of Rho A. Mutations within the carboxylate-binding loop of PDZ-RhoGEF result in changes in cell morphology and actin organization which is likely due to its interaction with MAP-1A (MAP1 light chain LC2). PDZ-RhoGEF also plays a role in B plexin-mediated activation of Rho/Rho kinase signaling, which is implicated in the regulation of axon guidance and cell migration.

## REFERENCES

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- Fukuhara, S., et al. 1999. A novel PDZ domain containing guanine nucleotide exchange factor links heterotrimeric G proteins to Rho. *J. Biol. Chem.* 274: 5868-5879.
- Garrard, S.M., et al. 2001. Expression, purification, and crystallization of the RGS-like domain from the Rho nucleotide exchange factor, PDZ-RhoGEF, using the surface entropy reduction approach. *Protein Expr. Purif.* 21: 412-416.
- Driessens, M.H., et al. 2002. B plexins activate Rho through PDZ-RhoGEF. *FEBS Lett.* 529: 168-172.
- Oleksy, A., et al. 2004. Preliminary crystallographic analysis of the complex of the human GTPase RhoA with the DH/PH tandem of PDZ-RhoGEF. *Acta Crystallogr. D Biol. Crystallogr.* 60: 740-742.
- Longhurst, D.M., et al. 2006. Interaction of PDZ-RhoGEF with microtubule-associated protein 1 light chains: link between microtubules, actin cytoskeleton, and neuronal polarity. *J. Biol. Chem.* 281: 12030-12040.

## CHROMOSOMAL LOCATION

Genetic locus: ARHGEF11 (human) mapping to 1q23.1.

## PRODUCT

PDZ-RhoGEF 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 PDZ-RhoGEF siRNA (h): sc-45823 and PDZ-RhoGEF shRNA Plasmid (h): sc-45823-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

PDZ-RhoGEF shRNA (h) Lentiviral Particles is recommended for the inhibition of PDZ-RhoGEF 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

PDZ-RhoGEF (N-14): sc-46234 is recommended as a control antibody for monitoring of PDZ-RhoGEF 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 PDZ-RhoGEF gene expression knockdown using RT-PCR Primer: PDZ-RhoGEF (h)-PR: sc-45823-PR (20  $\mu$ l, 427 bp). Annealing temperature for the primers should be  $55-60^\circ$  C and the extension temperature should be  $68-72^\circ$  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.

## 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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