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ARAP1 shRNA (h) Lentiviral Particles: sc-44444-V

BACKGROUND

ARAP1 (ARFGAP, Rho GAP, Ankyrin repeat and pleckstrin homology domains-containing protein 1), also designated Centaurin $\delta 2$ (CENTD2), contains ARF GAP, Rho GAP, Ankyrin repeat, RAS-associating and pleckstrin homology domains. ARAP1 displays Rho GAP and phosphatidylinositol (3,4,5) trisphosphate (PIP3)-dependent ARFGAP activity. It associates with the Golgi, and the ARF GAP activity mediates changes in the Golgi and the formation of filopodia. The Rho GAP activity may mediate cell rounding and loss of stress fibers. At least three transcript variants encoding different isoforms have been found for this gene. ARAP1 can associate with G Protein receptor Angiotensin 1 (AT1) and influences recycling of the AT1 receptor to the plasma membrane. ARAP1 transcript levels are abundant in ovary, lung, liver and kidney. Northern blots indicate an ubiquitous 5.5 kb ARAP1 transcript and an additional 7 kb transcript present in heart and skeletal muscle.

REFERENCES

1. Nagase, T., et al. 1998. Prediction of the coding sequences of unidentified human genes. XI. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. DNA Res. 5: 277-286.
2. Jacques, K.M., et al. 2002. ARF1 dissociates from the clathrin adaptor GGA prior to being inactivated by ARF GTPase-activating proteins. J. Biol. Chem. 277: 47235-47241.
3. Miura, K., et al. 2002. ARAP1: a point of convergence for ARF and Rho signaling. Mol. Cell 9: 109-119.
4. Santy, L.C., et al. 2002. GTPase signaling: bridging the GAP between ARF and Rho. Curr. Biol. 12: R360-362.
5. Guo, D.F., et al. 2003. Type 1 angiotensin II receptor-associated protein ARAP1 binds and recycles the receptor to the plasma membrane. Biochem. Biophys. Res. Commun. 310: 1254-1265.

CHROMOSOMAL LOCATION

Genetic locus: ARAP1 (human) mapping to 11q13.4.

PRODUCT

ARAP1 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 μ l frozen stock containing 1.0×10^6 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 ARAP1 siRNA (h): sc-44444 and ARAP1 shRNA Plasmid (h): sc-44444-SH as alternate gene silencing products.

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.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

APPLICATIONS

ARAP1 shRNA (h) Lentiviral Particles is recommended for the inhibition of ARAP1 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

ARAP1 (A-3): sc-393138 is recommended as a control antibody for monitoring of ARAP1 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 goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ARAP1 gene expression knockdown using RT-PCR Primer: ARAP1 (h)-PR: sc-44444-PR (20 μ l). 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.