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AGAP1 shRNA (h) Lentiviral Particles: sc-44443-V

BACKGROUND

ARFGAP with GTP-binding protein-like, Ankyrin repeat and pleckstrin homology domains 1 (AGAP1), also designated Centaurin γ 2 (CENTG2), is a member of the ADP ribosylation factor family of ARF6 GTPase-activating proteins (GAP). GAPs are important regulators of ARF function by controlling ARFs return to its inactive state. AGAP1, which is endosome-associated and phosphoinositide-dependent, regulates the adapter protein 3 (AP-3)-dependent trafficking of proteins in the endosomal-lysosomal system. The protein associates with the endocytic compartment in the cytoplasm and has an effect on the Actin cytoskeleton. Overexpression of AGAP1 induces a loss of Actin stress fibers. AGAP1 is related to ACAP1 and ASAP1, and all three proteins are similarly expressed in fibroblast cells such as NIH/3T3.

REFERENCES

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CHROMOSOMAL LOCATION

Genetic locus: AGAP1 (human) mapping to 2q37.2.

PRODUCT

AGAP1 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 AGAP1 siRNA (h): sc-44443 and AGAP1 shRNA Plasmid (h): sc-44443-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

AGAP1 shRNA (h) Lentiviral Particles is recommended for the inhibition of AGAP1 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

AGAP1 (N-15): sc-47786 is recommended as a control antibody for monitoring of AGAP1 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 AGAP1 gene expression knockdown using RT-PCR Primer: AGAP1 (h)-PR: sc-44443-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.