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## Produktinformation



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# RAP shRNA (h) Lentiviral Particles: sc-44068-V

## BACKGROUND

Members of the LDL receptor gene family, including LDLR (low density lipoprotein receptor), LRP (low density lipoprotein related protein), Megalin (also designated GP330), VLDLR (very low density lipoprotein receptor) and ApoER2, are characterized by a cluster of cysteine-rich class A repeats, epidermal growth factor (EGF)-like repeats, YWTD repeats and an O-linked sugar domain. LRP, also designated  $\alpha$ 2-Macroglobulin receptor, is an endocytic receptor that mediates the uptake of at least 15 ligands, including  $\alpha$ 2-Macroglobulin and apoE. LRP is cleaved into a membrane subunit and an extracellular subunit, which remain non-covalently associated. Proper folding and trafficking of LRP is facilitated by the receptor-associated protein (RAP), a molecular chaperone. The uptake of all known ligands through LRP can be blocked by RAP, which induces a conformational change in the receptor that renders it unable to bind ligands. LRP, which is expressed in brain, liver and lung, is also implicated in Alzheimer's disease (AD), as the human LRP gene localizes to a potential AD locus on chromosome 12.

## REFERENCES

1. Vash, B., et al. 1998. Three complement-type repeats of the low-density lipoprotein receptor-related protein define a common binding site for RAP, PAI-1 and lactoferrin. *Blood* 92: 3277-3285.
2. Trommsdorff, M., et al. 1999. Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and apoE receptor 2. *Cell* 97: 689-701.
3. Mikhailenko, I., et al. 1999. Functional domains of the very low density lipoprotein receptor: molecular analysis of ligand binding and acid-dependent ligand dissociation mechanisms. *J. Cell Sci.* 112: 3269-3281.
4. Neels, J.G., et al. 1999. The second and fourth cluster of class A cysteine-rich repeats of the low density lipoprotein receptor-related protein share ligand-binding properties. *J. Biol. Chem.* 274: 31305-31311.
5. Kang, D.E., et al. 2000. Modulation of Amyloid b-protein clearance and Alzheimer's disease susceptibility by the LDL receptor-related protein pathway. *J. Clin. Invest.* 106: 1159-1166.

## CHROMOSOMAL LOCATION

Genetic locus: LRPAP1 (human) mapping to 4p16.3.

## PRODUCT

RAP 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 RAP siRNA (h): sc-44068 and RAP shRNA Plasmid (h): sc-44068-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.

## APPLICATIONS

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

RAP (F-20): sc-16450 is recommended as a control antibody for monitoring of RAP 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 RAP gene expression knockdown using RT-PCR Primer: RAP (h)-PR: sc-44068-PR (20  $\mu$ l, 597 bp). 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.

## 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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