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# Raptor shRNA (h) Lentiviral Particles: sc-44069-V

## BACKGROUND

Regulatory associated protein of FRAP, also designated Raptor, is a binding partner for mammalian target of Rapamycin kinase (FRAP) and is essential for FRAP signaling *in vivo*. Raptor binding to FRAP is critical for FRAP-catalyzed substrate phosphorylation of 4E-BP1. The Raptor-FRAP complex is nutrient-sensitive and is important for a mechanism by which cells coordinate cell growth and size with changing environmental conditions. Raptor serves as a negative regulator of FRAP kinase activity under nutrient-deprived conditions and is an important component in the FRAP pathway. Raptor is highly expressed in skeletal muscle and to a lesser extent in brain, kidney, lung and placenta.

## REFERENCES

1. Hara, K., et al. 2002. Raptor, a binding partner of target of Rapamycin (TOR), mediates TOR action. *Cell* 110: 177-189.
2. Nojima, H., et al. 2003. The mammalian target of Rapamycin (mTOR) partner, Raptor, binds the mTOR substrates p70 S6 kinase and 4E-BP1 through their TOR signaling (TOS) motif. *J. Biol. Chem.* 278: 15461-15464.
3. Yonezawa, K., et al. 2004. Raptor, a binding partner of target of Rapamycin. *Biochem. Biophys. Res. Commun.* 313: 437-441.
4. Kim, D.H. and Sabatini, D.M. 2004. Raptor and mTOR: subunits of a nutrient-sensitive complex. *Curr. Top. Microbiol. Immunol.* 279: 259-270.
5. Oshiro, N., et al. 2004. Dissociation of Raptor from mTOR is a mechanism of Rapamycin-induced inhibition of mTOR function. *Genes Cells* 9: 359-366.
6. Ali, S.M., et al. 2005. Structure of S6 kinase 1 determines whether Raptor-mTOR or Rictor-mTOR phosphorylates its hydrophobic motif site. *J. Biol. Chem.* 280: 19445-19448.
7. Sarbassov, D.D., et al. 2005. Redox regulation of the nutrient-sensitive Raptor-mTOR pathway and complex. *J. Biol. Chem.* 280: 39505-39509.
8. Gwinn, D.M., et al. 2008. AMPK phosphorylation of Raptor mediates a metabolic checkpoint. *Mol. Cell.* 30: 214-226.

## CHROMOSOMAL LOCATION

Genetic locus: KIAA1303 (human) mapping to 17q25.3.

## PRODUCT

Raptor 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 Raptor siRNA (h): sc-44069 and Raptor shRNA Plasmid (h): sc-44069-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

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

Raptor (N-19): sc-27744 is recommended as a control antibody for monitoring of Raptor 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 Raptor gene expression knockdown using RT-PCR Primer: Raptor (h)-PR: sc-44069-PR (20  $\mu$ l, 533 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.

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