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p70 S6 kinase α shRNA (h2) Lentiviral Particles: sc-44318-V

BACKGROUND

In studies to elucidate key regulatory pathways in signal transduction, several protein serine/threonine (Ser/Thr) kinases have been identified, including two distinct families of 40S ribosomal protein S6 Ser/Thr kinases present in somatic animal cells, designated p70 S6 kinase and p90 Rsk kinase. p90 Rsk kinase is maximally activated within minutes of addition of growth factors or phorbol ester to cultured cells followed by activation of p70 S6 kinase. Both enzymes are regulated by serine/threonine phosphorylation, suggesting that specific kinases may exist upstream in the signaling pathway that regulate these kinases. In fact, evidence suggests that one such family of activating enzymes includes the members of the ERK MAP kinase family. The ERK MAP kinases are, in turn, regulated by phosphorylation at threonine and tyrosine residues by a protein kinase designated MEK.

REFERENCES

1. Alcorta, D.A., et al. 1989. Sequence and expression of chicken and mouse rsk: homologs of *Xenopus laevis* ribosomal S6 kinase. *Mol. Cell. Biol.* 9: 3850-3859.
2. Pelech, S.L., et al. 1990. Protein kinase cascades in meiotic and mitotic cell cycle control. *Biochem. Cell. Biol.* 68: 1297-1330.
3. Sweet, L.J., et al. 1990. Identification of mitogen-responsive ribosomal protein S6 kinase pp90rsk, a homolog of *Xenopus* S6 kinase II, in chicken embryo fibroblasts. *Mol. Cell. Biol.* 10: 2413-2417.
4. Banerjee, P., et al. 1990. Molecular structure of a major Insulin/mitogen-activated 70-kDa S6 protein kinase. *Proc. Natl. Acad. Sci. USA* 87: 8550-8554.

CHROMOSOMAL LOCATION

Genetic locus: RPS6KB1 (human) mapping to 17q23.1.

PRODUCT

p70 S6 kinase α shRNA (h2) 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 p70 S6 kinase α siRNA (h2): sc-44318 and p70 S6 kinase α shRNA Plasmid (h2): sc-44318-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

p70 S6 kinase α shRNA (h2) Lentiviral Particles is recommended for the inhibition of p70 S6 kinase α 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

p70 S6 kinase α (H-9): sc-8418 is recommended as a control antibody for monitoring of p70 S6 kinase α 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 p70 S6 kinase α gene expression knockdown using RT-PCR Primer: p70 S6 kinase α (h2)-PR: sc-44318-PR (20 μ l, 531 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.