SANTA CRUZ BIOTECHNOLOGY, INC.

p70 S6 kinase α siRNA (h2): sc-44318

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. Kozma, S.C., et al. 1990. Cloning of the mitogen-activated S6 kinase from rat liver reveals an enzyme of the second messenger subfamily. Proc. Natl. Acad. Sci. USA 87: 7365-7369.
5. 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.
6. Erikson, R.L. 1991. Structure, expression, and regulation of protein kinases involved in the phosphorylation of ribosomal protein S6. J. Biol. Chem. 266: 6007-6010.
7. Chen, R.H., et al. 1992. Nuclear localization and regulation of Erk- and Rsk-encoded protein kinases. Mol. Cell. Biol. 12: 915-927.

CHROMOSOMAL LOCATION

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

PRODUCT

p70 S6 kinase α siRNA (h2) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see p70 S6 kinase α shRNA Plasmid (h2): sc-44318-SH and p70 S6 kinase α shRNA (h2) Lentiviral Particles: sc-44318-V as alternate gene silencing products.

For independent verification of p70 S6 kinase α (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44318A, sc-44318B and sc-44318C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

p70 S6 kinase α siRNA (h2) is recommended for the inhibition of p70 S6 kinase α expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor gene expression knockdown using RT-PCR Primer: p70 S6 kinase α (h2)-PR: sc-44318-PR (20 µl, 531 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

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