

Ksr-1 siRNA (m): sc-35763

BACKGROUND

Several serine/threonine protein kinases have been implicated as intermediates in signal transduction pathways. These include ERK/MAP kinases, ribosomal S6 kinase (Rsk) and Raf-1. Raf-1 has intrinsic kinase activity towards serine/threonine residues and is widely expressed in many tissue types and cell lines. Raf-1 activation is dependent on the small molecular weight GTPase Ras, but the means by which this activation occurs is poorly understood. Two proteins putatively involved in this process are Ksr-1 and Tak1. Ksr-1 (kinase suppressor of Ras) is a novel Raf-related protein kinase whose function is required for Ras signal transduction. Whether Ksr-1 lies directly downstream of Ras or acts in a parallel pathway is not yet known. Tak1 (TGF β -activated kinase) has been shown to participate in the activation of the MAP kinase family in response to TGF β stimulation.

REFERENCES

1. Huleihel, M., et al. 1986. Characterization of murine A-raf, a new oncogene related to the v-raf oncogene. *Mol. Cell. Biol.* 6: 2655-2662.
2. Ray, L.B., et al. 1988. Insulin-stimulated microtubule-associated protein kinase is phosphorylated on tyrosine and threonine *in vivo*. *Proc. Natl. Acad. Sci. USA* 85: 3753-3757.
3. Morrison, D.K., et al. 1988. Signal transduction from membrane to cytoplasm: growth factors and membrane-bound oncogene products increase Raf-1 phosphorylation and associated protein kinase activity. *Proc. Natl. Acad. Sci. USA* 85: 8855-8859.
4. Pelech, S.L., et al. 1990. Protein kinase cascades in meiotic and mitotic cell cycle control. *Biochem. Cell Biol.* 68: 1297-1330.
5. Downward, J. 1995. KSR: a novel player in the RAS pathway. *Cell* 83: 831-834.
6. Therrien, M., et al. 1995. KSR, a novel protein kinase required for RAS signal transduction. *Cell* 83: 879-888.
7. Sundaram, M. and Han, M. 1995. The *C. elegans* ksr-1 gene encodes a novel Raf-related kinase involved in Ras-mediated signal transduction. *Cell* 83: 889-901.

CHROMOSOMAL LOCATION

Genetic locus: Ksr1 (mouse) mapping to 11 B5.

PRODUCT

Ksr-1 siRNA (m) 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Ksr-1 shRNA Plasmid (m): sc-35763-SH and Ksr-1 shRNA (m) Lentiviral Particles: sc-35763-V as alternate gene silencing products.

For independent verification of Ksr-1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35763A, sc-35763B and sc-35763C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Ksr-1 siRNA (m) is recommended for the inhibition of Ksr-1 expression in mouse 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 μ M in 66 μ l. 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

Ksr-1 (E-5): sc-515924 is recommended as a control antibody for monitoring of Ksr-1 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Ksr-1 gene expression knockdown using RT-PCR Primer: Ksr-1 (m)-PR: sc-35763-PR (20 μ l, 455 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.