SANTA CRUZ BIOTECHNOLOGY, INC.

Ksr-2 siRNA (m): sc-60902

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The Power to Question

BACKGROUND

Kinase suppressor of Ras (Ksr) and MAP kinase kinase kinase (MEKK3) are integral members of the MAP kinase pathway. Ksr is a conserved protein that positively regulates Ras signaling and may function as a scaffold for Raf, MEK and ERK. There are two types of Ksr proteins: Ksr-1 and Ksr-2. These two are individually necessary for a few specific Ras-dependent processes, but they are required together for most aspects of Ras-mediated signaling. Ksr-2 plays a key role in Ras-mediated signaling during germline meiotic progression and functions redundantly with Ksr-1 during the development of the excretory system pathway, hermaphrodite vulva, and male spicules. Ksr-2 also functions as a negative regulator of the MEKK3-mediated activation of the MAP kinase pathways (specifically ERK and JNK) and of the NFκB pathways, and it simultaneously inhibits MEKK3-mediated Il-8 production.

REFERENCES

1. Sundaram, M., et al. 1996. The *C. elegans* Ksr-1 gene encodes a novel Raf-related kinase involved in Ras-mediated signal transduction. Cell 83: 889-901.
2. Denouel-Galy, A., et al. 1998. Murine Ksr interacts with MEK and inhibits Ras-induced transformation. Curr. Biol. 8: 46-55.
3. Joneson, T., et al. 1998. Kinase suppressor of Ras inhibits the activation of extracellular ligand-regulated (ERK) mitogen-activated protein (MAP) kinase by growth factors, activated Ras, and Ras effectors. J. Biol. Chem. 273: 7743-7748.
4. Yu, W., et al. 1998. Regulation of the MAP kinase pathway by mammalian Ksr through direct interaction with MEK and ERK. Curr. Biol. 8: 56-64.
5. Sugimoto, T., et al. 1998. The kinase suppressor of by uncoupling Elk-1 phosphorylation from MAP kinase activation. EMBO J. 17: 1717-1727.
6. Stewart, S., et al. 1999. Kinase suppressor of Ras forms a multiprotein signaling complex and modulates MEK localization. Mol. Cell. Biol. 19: 5523-5534.
7. Ohmachi, M., et al. 2002. *C. elegans* ksr-1 and ksr-2 have both unique and redundant functions and a required for MPK-1 ERK phosphorylation. Curr. Biol. 12: 427-433.

CHROMOSOMAL LOCATION

Genetic locus: Ksr2 (mouse) mapping to 5 F.

PRODUCT

Ksr-2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Ksr-2 shRNA Plasmid (m): sc-60902-SH and Ksr-2 shRNA (m) Lentiviral Particles: sc-60902-V as alternate gene silencing products.

For independent verification of Ksr-2 (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-60902A, sc-60902B and sc-60902C.

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

Ksr-2 siRNA (m) is recommended for the inhibition of Ksr-2 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 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

Ksr-2 (K75): sc-100421 is recommended as a control antibody for monitoring of Ksr-2 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 Ksr-2 gene expression knockdown using RT-PCR Primer: Ksr-2 (m)-PR: sc-60902-PR (20 µl). 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.