

Krs-2 siRNA (r): sc-270632

BACKGROUND

Sterile-20 (Ste20) is a serine/threonine kinase in *Saccharomyces cerevisiae* that is involved in relaying signals from G protein-coupled receptors to cytosolic MAP kinase cascades. Mammalian protein kinases that display sequence similarity to Ste20 are divided into two groups, the PAK subfamily and the GCK subfamily. The PAK subfamily members contain a C-terminal catalytic domain and an N-terminal regulatory domain with a p21Rac/Cdc42-binding site, and these kinases can activate both p38 MAPK and JNK. The GCK subfamily members contain a C-terminal regulatory domain and an N-terminal catalytic domain, and they have diverse roles in many pathways, including the activation of ERK, JNK, p38 MAPK, and caspase-3. The mammalian Ste20-like kinases (MST kinases), also known as Krs proteins, are members of the GCK subfamily. Ksr-1 (MST-2) and Ksr-2 (MST-1) are both direct substrates of caspase-3 that accelerate caspase-3 activation. MST-3 is ubiquitously expressed in mammalian tissue and can phosphorylate exogenous substrates as well as itself. MST-4 is highly expressed in placenta, thymus, and peripheral blood leukocytes, and it specifically activates ERK.

REFERENCES

1. Leberer, E., et al. 1992. The protein kinase homologue Ste20p is required to link the yeast pheromone response G-protein $\beta\gamma$ subunits to downstream signalling components. *EMBO J.* 11: 4815-4824.
2. Schinkmann, K., et al. 1997. Cloning and characterization of a human STE20-like protein kinase with unusual cofactor requirements. *J. Biol. Chem.* 272: 28695-28703.
3. Raitt, D., et al. 2000. Yeast Cdc42 GTPase and Ste20 PAK-like kinase regulate Sho1-dependent activation of the Hog1 MAPK pathway. *EMBO J.* 17: 4623-4631.
4. Zhou, T.H., et al. 2000. Identification of a human brain-specific isoform of mammalian STE20-like kinase 3 that is regulated by cAMP-dependent protein kinase. *J. Biol. Chem.* 275: 2513-2519.
5. Lin, J.L., et al. 2001. MST4, a new Ste20-related kinase that mediates cell growth and transformation via modulating ERK pathway. *Oncogene* 20: 6559-6569.

CHROMOSOMAL LOCATION

Genetic locus: Stk4 (rat) mapping to 3q42.

PRODUCT

Krs-2 siRNA (r) 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 Krs-2 shRNA Plasmid (r): sc-270632-SH and Krs-2 shRNA (r) Lentiviral Particles: sc-270632-V as alternate gene silencing products.

For independent verification of Krs-2 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270632A, sc-270632B and sc-270632C.

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

Krs-2 siRNA (r) is recommended for the inhibition of Krs-2 expression in rat 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

Krs-2 (H-8): sc-515051 is recommended as a control antibody for monitoring of Krs-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-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 Krs-2 gene expression knockdown using RT-PCR Primer: Krs-2 (r)-PR: sc-270632-PR (20 μ l). 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.