

GLK siRNA (m): sc-39242

BACKGROUND

Several mammalian kinases have been identified which exhibit sequence similarity to the *Saccharomyces cerevisiae* serine/threonine kinase Ste20. Ste20 is involved in relaying signals from G protein-coupled receptors to cytosolic MAP kinase cascades, and it lies upstream of a MAP kinase kinase. Mammalian Ste20-like kinases include GLK, KHS, NIK, YSK1, HPK1, Krs-1, Krs-2 and human GC kinase. GLK (for GC-like kinase) is an 885 amino acid protein that shares a high degree of homology with GC kinase and HPK1. Like many of the Ste20-like kinases, GLK specifically activates the JNK pathway. Epistasis studies with dominant negative mutants of MEKK1 suggest that GLK functions upstream of MEKK1 in the JNK signaling pathway.

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. Wu, C., et al. 1995. Molecular characterization of Ste20p, a potential mitogen-activated protein or extracellular signal-regulated kinase kinase (MEK) kinase from *Saccharomyces cerevisiae*. *J. Biol. Chem.* 270: 15984-15992.
3. Hu, M.C., et al. 1996. Human HPK1, a novel human hematopoietic progenitor kinase that activates the JNK/SAPK kinase cascade. *Genes Dev.* 10: 2251-2264.
4. Su, Y.C., et al. 1997. NIK is a new Ste20-related kinase that binds NCK and MEKK1 and activates the SAPK/JNK cascade via a conserved regulatory domain. *EMBO J.* 16: 1279-1290.
5. Diener, K., et al. 1997. Activation of the c-Jun N-terminal kinase pathway by a novel protein kinase related to human germinal center kinase. *Proc. Natl. Acad. Sci. USA* 94: 9687-9692.

CHROMOSOMAL LOCATION

Genetic locus: Map4k3 (mouse) mapping to 17 E3.

PRODUCT

GLK 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 GLK shRNA Plasmid (m): sc-39242-SH and GLK shRNA (m) Lentiviral Particles: sc-39242-V as alternate gene silencing products.

For independent verification of GLK (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-39242A, sc-39242B and sc-39242C.

PROTOCOLS

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

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

GLK siRNA (m) is recommended for the inhibition of GLK 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

GLK (C-6): sc-515064 is recommended as a control antibody for monitoring of GLK 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 GLK gene expression knockdown using RT-PCR Primer: GLK (m)-PR: sc-39242-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.