

HGK siRNA (m): sc-39244

BACKGROUND

HGK (HPK/GC kinase-like kinase), also known as mitogen-activated protein kinase kinase kinase kinase 4, MAPK/ERK kinase kinase kinase 4, MEKKK 4 or NCK-interacting kinase (NIK), is a member of the serine/threonine kinase subfamily, Ste20. This subfamily is comprised of several mammalian kinases which exhibit sequence similarity to the *Saccharomyces cerevisiae* serine/threonine kinase, Ste20. Members of this subfamily include KHS, GLK, YSK1, HPK1, Krs-1, Krs-2, GC kinase and HGK. HGK, like many of the Ste20-like kinases, has been shown to activate the SAPK/JNK stress response pathway. HGK interacts with MEK kinase-1 and is thought to act upstream of MEK kinase-1 in the SAPK/JNK signaling pathway. Both the kinase domain and the C-terminal regulatory domain of HGK are required for full activation.

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 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.

CHROMOSOMAL LOCATION

Genetic locus: Map4k4 (mouse) mapping to 1 B.

PRODUCT

HGK 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 HGK shRNA Plasmid (m): sc-39244-SH and HGK shRNA (m) Lentiviral Particles: sc-39244-V as alternate gene silencing products.

For independent verification of HGK (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-39244A, sc-39244B and sc-39244C.

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

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

HGK (5-12): sc-100445 is recommended as a control antibody for monitoring of HGK 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 HGK gene expression knockdown using RT-PCR Primer: HGK (m)-PR: sc-39244-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Wu, C., et al. 2019. MAP4K4 activation mediates motor neuron degeneration in amyotrophic lateral sclerosis. *Cell Rep.* 26: 1143-1156.
2. Zou, Z., et al. 2021. MAP4K4 induces early blood-brain barrier damage in a murine subarachnoid hemorrhage model. *Neural Regen. Res.* 16: 325-332.

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.