

HIPK2 siRNA (m): sc-39051

BACKGROUND

The homeodomain-interacting protein kinase (HIPK) family, which includes HIPK1, HIPK2, HIPK3, contains a conserved protein kinase domain as well as a separate domain that interacts with homeoproteins. HIPK2, the most highly characterized family member, is thought to act as a co-repressor of homeodomain transcription factors as HIPK2 has been shown to enhance the DNA binding of the NK-3 homeoprotein *in vitro*. It is regulated by a posttranslational modification of a ubiquitin-like protein, SUMO-1, via covalent bonding to a lysine residue on HIPK2. This is similar to the binding of SUMO-1 to PML and Sp100. The conjugation of SUMO-1 is thought to direct each of these proteins to nuclear bodies (NB's), which appear to play a role in autoimmunity and viral protection. HIPK2 is the first protein kinase to be directed to nuclear bodies in response to ubiquitin-like modification.

REFERENCES

1. Kim, Y.H., et al. 1998. Homeodomain-interacting protein kinases, a novel family of co-repressors for homeodomain transcription factors. *J. Biol. Chem.* 273: 25875-25879.
2. Schwarz, S.E., et al. 1998. The ubiquitin-like proteins SMT3 and SUMO-1 are conjugated by the UBC9 E2 enzyme. *Proc. Natl. Acad. Sci. USA* 95: 560-564.
3. Kim, Y.H., et al. 1999. Covalent modification of the homeodomain-interacting protein kinase 2 (HIPK2) by the ubiquitin-like protein SUMO-1. *Proc. Natl. Acad. Sci. USA* 96: 12350-12355.
4. Muller, S., et al. 1999. Viral immediate-early proteins abrogate the modification of SUMO-1 of PML and Sp100 proteins, correlating with nuclear body disruption. *J. Virol.* 73: 5137-5143.

CHROMOSOMAL LOCATION

Genetic locus: *Hipk2* (mouse) mapping to 6 B1.

PRODUCT

HIPK2 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 HIPK2 shRNA Plasmid (m): sc-39051-SH and HIPK2 shRNA (m) Lentiviral Particles: sc-39051-V as alternate gene silencing products.

For independent verification of HIPK2 (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-39051A, sc-39051B and sc-39051C.

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

HIPK2 siRNA (m) is recommended for the inhibition of HIPK2 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

HIPK2 (F-189): sc-100383 is recommended as a control antibody for monitoring of HIPK2 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 HIPK2 gene expression knockdown using RT-PCR Primer: HIPK2 (m)-PR: sc-39051-PR (20 μ l, 494 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Boucher, M.J., et al. 2009. The homeodomain-interacting protein kinase 2 regulates Insulin promoter factor-1/pancreatic duodenal homeobox-1 transcriptional activity. *Endocrinology* 150: 87-97.

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.