

JIP-1 siRNA (h): sc-35722

BACKGROUND

c-Jun N^H₂-terminal kinases (JNKs) are distant members of the MAP kinase family. JNK1 is activated by dual phosphorylation at a Thr-Pro-Tyr motif in response to ultraviolet (UV) light, and it functions to phosphorylate c-Jun at amino-terminal serine regulatory sites, Ser 63 and Ser 73, resulting in transcriptional activation. Two additional JNK family members have been identified as JNK2 and JNK3. JIP-1 (for JNK interacting protein-1) has been identified as a cytoplasmic inhibitor of JNK that retains JNK in the cytoplasm, thereby inhibiting JNK-regulated gene expression. Evidence suggests that JNK1 and JNK2 bind to JIP-1 with greater affinity than to ATF-2 and c-Jun, which are targets of the JNK signaling pathway. JIP-1 contains an amino-terminal JNK binding domain and a carboxy-terminal SH3 domain. ATF-2 and c-Jun also contain the JNK binding domain and are thought to compete with JIP-1 for JNK binding. Multiple splice variants of JIP-1, including JIP-1b, JIP-1c (also designated islet-brain 1 or IB-1), JIP-2a, JIP-2b and JIP-3, have been identified in brain.

REFERENCES

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2. Smeal, T., et al. 1992. Oncoprotein-mediated signalling cascade stimulates c-Jun activity by phosphorylation of Serines 63 and 73. *Mol. Cell. Biol.* 12: 3507-3512.
3. Derijard, B., et al. 1994. JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* 76: 1025-1037.
4. Kyriakis, J.M., et al. 1994. The stress-activated protein kinase subfamily of c-Jun kinases. *Nature* 369: 156-160.
5. Davis, R.J. 1995. Transcriptional regulation by MAP kinases. *Mol. Reprod. Dev.* 42: 459-467.
6. Dickens, M., et al. 1997. A cytoplasmic inhibitor of the JNK signal transduction pathway. *Science* 277: 693-696.
7. Kim, I.J., et al. 1999. Molecular cloning of multiple splicing variants of JIP-1 preferentially expressed in brain. *J. Neurochem.* 72: 1335-1343.

CHROMOSOMAL LOCATION

Genetic locus: MAPK8IP1 (human) mapping to 11p11.2.

PRODUCT

JIP-1 siRNA (h) 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 JIP-1 shRNA Plasmid (h): sc-35722-SH and JIP-1 shRNA (h) Lentiviral Particles: sc-35722-V as alternate gene silencing products.

For independent verification of JIP-1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35722A, sc-35722B and sc-35722C.

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

JIP-1 siRNA (h) is recommended for the inhibition of JIP-1 expression in human 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

JIP-1 (2J8): sc-53552 is recommended as a control antibody for monitoring of JIP-1 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 JIP-1 gene expression knockdown using RT-PCR Primer: JIP-1 (h)-PR: sc-35722-PR (20 μ l, 500 bp). 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.