

JIP-3 siRNA (h2): sc-43368

BACKGROUND

The JNK-interacting proteins (JIPs) are a family of scaffold proteins that mediate JNK signaling by organizing specific components of the MAPK cascade together to form a functional JNK signaling molecule. JIP-3 (JNK-interacting protein 3), also known as JSAP1 or MAPK8IP3 (mitogen-activated protein kinase 8-interacting protein 3), is a 1,336 amino acid protein that localizes to the cytoplasm and belongs to the JIP family. Expressed in a variety of tissues, including brain and heart, JIP-3 forms homo- or heterooligomeric complexes that can interact with several components of the JNK signaling pathway, thereby functioning as a regulator of kinesin-dependent axonal transport that may also play a role in scaffold formation within neuronal cells. Human JIP-3, which may be phosphorylated upon DNA damage, shares 69% similarity with its mouse counterpart, suggesting a conserved role between species. Multiple isoforms of JIP-3 exist due to alternative splicing events.

REFERENCES

1. Ito, M., et al. 1999. JSAP1, a novel Jun N-terminal protein kinase (JNK)-binding protein that functions as a scaffold factor in the JNK signaling pathway. *Mol. Cell. Biol.* 19: 7539-7548.
2. Bowman, A.B., et al. 2000. Kinesin-dependent axonal transport is mediated by the Sunday driver (SYD) protein. *Cell* 103: 583-594.
3. Kelkar, N., et al. 2000. Interaction of a mitogen-activated protein kinase signaling module with the neuronal protein JIP3. *Mol. Cell. Biol.* 20: 1030-1043.
4. Akechi, M., et al. 2001. Expression of JNK cascade scaffold protein JSAP1 in the mouse nervous system. *Neurosci. Res.* 39: 391-400.
5. Matsuura, H., et al. 2002. Phosphorylation-dependent scaffolding role of JSAP1/JIP3 in the ASK1-JNK signaling pathway. A new mode of regulation of the MAP kinase cascade. *J. Biol. Chem.* 277: 40703-40709.
6. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 605431. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
7. Morita, N., et al. 2004. N-terminal kinase, and c-Src are activated in human aortic smooth muscle cells by pressure stress. *Mol. Cell. Biochem.* 262: 71-78.

CHROMOSOMAL LOCATION

Genetic locus: MAPK8IP3 (human) mapping to 16p13.3.

PRODUCT

JIP-3 siRNA (h2) 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-3 shRNA Plasmid (h2): sc-43368-SH and JIP-3 shRNA (h2) Lentiviral Particles: sc-43368-V as alternate gene silencing products.

For independent verification of JIP-3 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-43368A, sc-43368B and sc-43368C.

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-3 siRNA (h2) is recommended for the inhibition of JIP-3 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-3 (F-6): sc-46663 is recommended as a control antibody for monitoring of 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-3 gene expression knockdown using RT-PCR Primer: JIP-3 (h2)-PR: sc-43368-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.