

JIP-4 siRNA (m): sc-62514

BACKGROUND

JIP-4 (c-Jun-amino-terminal kinase-interacting protein 4, mitogen-activated protein kinase 8-interacting protein 4, Sunday driver 1) is a 1,321 amino acid protein encoded by the human gene SPAG9. It contains a large N-terminal extracellular domain, a short transmembrane helical domain, and a cytoplasmic domain. There are six N-glycosylation sites, several phosphorylation sites for cAMP/cGMP-dependent protein kinase, protein kinase C, and casein kinase II, and ten putative myristoylation sites. There is also a leucine zipper motif, with 6 leucine repeats, that may aid in dimerization since there is no upstream basic domain characteristic of DNA binding proteins. The JNK-interacting protein (JIP) group of scaffold proteins selectively mediates JNK signaling by aggregating specific components of the MAPK cascade to form a functional JNK signaling module. JIP-4 is a cytoplasmic, perinuclear protein that has eight known isoforms whose expression varies by tissue and disease state.

REFERENCES

- Shankar, S., et al. 1998. Cloning of a novel human testis mRNA specifically expressed in testicular haploid germ cells, having unique palindromic sequences and encoding a leucine zipper dimerization motif. *Biochem. Biophys. Res. Commun.* 243: 561-565.
- Bowman, A.B., et al. 2001. Kinesin-dependent axonal transport is mediated by the Sunday driver (SYD) protein. *Cell* 103: 583-594.
- Lee, C.M., et al. 2002. JLP: A scaffolding protein that tethers JNK/p38MAPK signaling modules and transcription factors. *Proc. Natl. Acad. Sci. USA* 99: 14189-14194.
- Yasuoka, H., et al. 2003. A novel protein highly expressed in testis is overexpressed in systemic sclerosis fibroblasts and targeted by autoantibodies. *J. Immunol.* 171: 6883-6890.
- Jagadeish, N., et al. 2005. Sperm associated antigen 9 (SPAG9): a new member of c-Jun NH₂-terminal kinase (JNK) interacting protein exclusively expressed in testis. *Keio J. Med.* 54: 66-71.
- Jagadeish, N., et al. 2005. Characterization of a novel human sperm-associated antigen 9 (SPAG9) having structural homology with c-Jun N-terminal kinase-interacting protein. *Biochem. J.* 389: 73-82.

CHROMOSOMAL LOCATION

Genetic locus: Spag9 (mouse) mapping to 11 D.

PRODUCT

JIP-4 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 JIP-4 shRNA Plasmid (m): sc-62514-SH and JIP-4 shRNA (m) Lentiviral Particles: sc-62514-V as alternate gene silencing products.

For independent verification of JIP-4 (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-62514A, sc-62514B and sc-62514C.

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-4 siRNA (m) is recommended for the inhibition of JIP-4 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

JIP-4 (H-8): sc-271492 is recommended as a control antibody for monitoring of JIP-4 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-4 gene expression knockdown using RT-PCR Primer: JIP-4 (m)-PR: sc-62514-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.