SANTA CRUZ BIOTECHNOLOGY, INC.

IRSp53 siRNA (m): sc-60864

BACKGROUND

The scaffolding protein Insulin receptor tyrosine kinase substrate p53 (IRSp53), a ubiquitous regulator of the Actin cytoskeleton, mediates filopodia formation under the control of Rho family GTPases. It is expressed in the cytoplasm and links small membrane-bound G proteins to cytoplasmic effector proteins. IRSp53 comprises a central SH3 domain, which binds to proline-rich regions of a wide range of Actin regulators, and a conserved N-terminal IRSp53/MIM homology domain (IMD) that harbors F-Actin-bundling activity. IRSp53 interacts with atrophin-1, the product of the dentatorubral-pallidoluysian atrophy (DRPLA) gene, which is associated with an autosomal dominant neurodegenerative disease. The IRSp53 protein also interacts with ENAH, BAI-1, Eps8, Shank 1, Shank 2, Shank 3, WAVE1, WAVE2, Tiam1 and Dia 1.

REFERENCES

1. Okamura-Oho, Y., et al. 1999. Dentatorubral-pallidoluysian atrophy protein INT region near polyglutamine with the SH3 domain of an Insulin receptor tyrosine kinase substrate. Hum. Mol. Genet. 8: 947-957.
2. Soltau, M., et al. 2002. The Insulin receptor substrate IRSp53 links postsynaptic Shank 1 to the small G protein Cdc42. Mol. Cell. Neurosci. 21: 575-583.
3. Miyahara, A., et al. 2003. Genomic structure and alternati kinase substrate of 53 kDa protein. J. Hum. Genet. 48: 410-414.
4. Funato, Y., et al. 2004. IRSp53/Eps8 complex is important for positive regulation of Rac and cancer cell motility/invasiveness. Cancer Res. 64: 5237-5244.
5. Choi, J. 2005. Regulation of Dendritic spine morphogenesis by Insulin receptor substrate 53, a downstream effector of Rac 1 and Cdc42 small GTPases. J. Neurosci. 25: 869-789.
6. Connolly, B.A., et al. 2005. Tiam1-IRSp53 complex formation directs specificity of Rac-mediated Actin cytoskeleton regulation. Mol. Cell. Biol. 25: 4602-4614.
7. Hori, K., et al. 2005. NMDA receptor-dependent synaptic translocation of Insulin receptor substrate p53 via protein kinase C signaling. J. Neurosci. 25: 2670-2681.

CHROMOSOMAL LOCATION

Genetic locus: Baiap2 (mouse) mapping to 11 E2.

PRODUCT

IRSp53 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see IRSp53 shRNA Plasmid (m): sc-60864-SH and IRSp53 shRNA (m) Lentiviral Particles: sc-60864-V as alternate gene silencing products.

For independent verification of IRSp53 (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-60864A, sc-60864B and sc-60864C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

IRSp53 siRNA (m) is recommended for the inhibition of IRSp53 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 µM in 66 µl. 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

IRSp53 (46): sc-136470 is recommended as a control antibody for monitoring of IRSp53 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 IRSp53 gene expression knockdown using RT-PCR Primer: IRSp53 (m)-PR: sc-60864-PR (20 µl, 581 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.