

IP3R-II siRNA (m): sc-35699

BACKGROUND

Inositol 1,4,5-triphosphate (IP3) functions as a second messenger for a myriad of extracellular stimuli including hormones, growth factors and neurotransmitters. Receptor tyrosine kinases indirectly increase the intracellular levels of IP3 through the activation of phospholipases such as phospholipase C (PLC), which convert phosphatidylinositol-4,5 bisphosphate into IP3 and diacylglycerol (DAG). The inositol 1,4,5-triphosphate receptor, IP3R, acts as an inositol triphosphate (IP3)-gated calcium release channel in a variety of cell types. Three IP3 receptor subtypes have been described and are designated IP3R-I, IP3R-II and IP3R-III. IP3R-I is the predominant IP3R subtype expressed in neuronal tissues and the central nervous system, but is also expressed at high levels in the liver.

REFERENCES

1. Blondel, O., et al. 1993. Sequence and functional characterization of a third inositol triphosphate receptor subtype, IP3R-3, expressed in pancreatic islets, kidney, gastrointestinal tract, and other tissues. *J. Biol. Chem.* 268: 11356-11363.
2. Raghu, P., et al. 1995. The inositol 1,4,5-triphosphate receptor expression in *Drosophila* suggests a role for IP3 signalling in muscle development and adult hemosensory functions. *Dev. Biol.* 171: 564-577.
3. Zhang, S.X., et al. 1995. *In situ* hybridization of mRNA expression for IP3 receptor and IP3-3-kinase in rat brain after transient focal cerebral ischemia. *Mol. Brain Res.* 32: 252-260.
4. Joseph, S.K., et al. 1995. Heterologomers of type-I and type-III inositol triphosphate receptors in WB rat liver epithelial cells. *J. Biol. Chem.* 270: 23310-23316.
5. Cameron, A.M., et al. 1995. Calcineurin associated with the inositol 1,4,5-trisphosphate receptor-FKBP12 complex modulates Ca²⁺ flux. *Cell* 83: 463-472.
6. Jayaraman, T., et al. 1996. Regulation of the inositol 1,4,5-trisphosphate receptor by tyrosine phosphorylation. *Science* 272: 1492-1494.
7. Khan, A.A., et al. 1996. Lymphocyte apoptosis: mediation by increased type 3 inositol 1,4,5-trisphosphate receptor. *Science* 273: 503-507.

CHROMOSOMAL LOCATION

Genetic locus: *Itp2* (mouse) mapping to 6 G3.

PRODUCT

IP3R-II siRNA (m) is a pool of 2 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 IP3R-II shRNA Plasmid (m): sc-35699-SH and IP3R-II shRNA (m) Lentiviral Particles: sc-35699-V as alternate gene silencing products.

For independent verification of IP3R-II (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-35699A and sc-35699B.

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

IP3R-II siRNA (m) is recommended for the inhibition of IP3R-II 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

IP3R-II (A-5): sc-398434 is recommended as a control antibody for monitoring of IP3R-II 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 IP3R-II gene expression knockdown using RT-PCR Primer: IP3R-II (m)-PR: sc-35699-PR (20 μ l, 544 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.