

IRS-4 siRNA (h): sc-35715

BACKGROUND

The Insulin receptor substrate (IRS) proteins are key components in signaling from the Insulin receptor. IRS-4 is the most recently characterized member of the IRS family and has an undefined *in vivo* function. Phosphorylated IRS-4 associates with phosphatidylinositol 3-kinase (PI3-kinase), involved in Insulin-stimulated DNA synthesis, GH-induced tyrosine phosphorylation of IRS-4 and nuclear translocation of Stat5. IRS-4 also associates with IRAS which, when overexpressed, enhances IRS-4-dependent Insulin stimulation of PI3-kinase. The IRS-4 protein exhibits a limited fiber type specific expression in heart and skeletal muscle tissue and has not yet been detected in any mouse or primary human tissue. The absence of IRS-4 in mice causes mild defects in growth, reproduction and glucose homeostasis, while overexpression of IRS-4 increases basal PI3-kinase activity and Akt phosphorylation. Defects in IRS-4-null mice may result from a lower overall blood glucose concentration.

REFERENCES

1. Fantin, V.R., et al. 2000. Mice lacking Insulin receptor substrate 4 exhibit mild defects in growth, reproduction and glucose homeostasis. *Am. J. Physiol. Endocrinol. Metab.* 278: E127-E133.
2. Tsuruzoe, K., et al. 2001. Insulin receptor substrate 3 (IRS-3) and IRS-4 impair IRS-1- and IRS-2-mediated signaling. *Mol. Cell. Biol.* 21: 26-38.
3. Sano, H., et al. 2002. Insulin receptor substrate 4 associates with the protein IRAS. *J. Biol. Chem.* 277: 19439-19447.
4. Schreyer, S., et al. 2003. Insulin receptor substrate-4 is expressed in muscle tissue without acting as a substrate for the Insulin receptor. *Endocrinology* 144: 1211-1218.
5. Urso, B., et al. 2003. IRS-4 mediated mitogenic signalling by Insulin and growth hormone in LB cells, a murine T-cell lymphoma devoid of IGF-1 receptors. *Cell. Signal.* 15: 385-394.

CHROMOSOMAL LOCATION

Genetic locus: IRS4 (human) mapping to Xq22.3.

PRODUCT

IRS-4 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 IRS-4 shRNA Plasmid (h): sc-35715-SH and IRS-4 shRNA (h) Lentiviral Particles: sc-35715-V as alternate gene silencing products.

For independent verification of IRS-4 (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-35715A, sc-35715B and sc-35715C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

IRS-4 siRNA (h) is recommended for the inhibition of IRS-4 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

IRS-4 (C-1): sc-373778 is recommended as a control antibody for monitoring of IRS-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 IRS-4 gene expression knockdown using RT-PCR Primer: IRS-4 (h)-PR: sc-35715-PR (20 μ l, 488 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.