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SANTA CRUZ BIOTECHNOLOGY, INC.

IRS-2 siRNA (r): sc-155988

BACKGROUND

IRS-2, originally described as 4PS, acts as a signaling intermediate downstream of the Insulin, IGF-1, IL-4, IL-9 and IL-13 receptors. In IRS-2-deficient mice, reduction in total PI 3-kinase activity by 30% and abolition of downstream activation of protein kinase C (PKC) ζ leads to the development of type 2 diabetes. Additionally, reconstitution with retroviral IRS-2 restores IRS-2/PI 3-kinase/PKC ζ signalling as well as glucose uptake. IRS-2 translocates to the nuclei of mouse embryo fibroblasts expressing the Insulin-like growth factor 1 receptor. Various mutations in the IGF-IR can result in an abrogation of or decrease in the translocation of IRS proteins to the nucleoli. IRS-2 is responsible for mitogen-activated protein kinase (MAPK) and protein kinase B (PKB) activation by Insulin and is the major adapter molecule linking the Insulin receptor to this step.

REFERENCES

1. Sun, X.J., et al. 1995. Role of IRS-2 in Insulin and cytokine signalling. Nature 377: 173-177.
2. Wang, L.M., et al. 1995. The Insulin receptor substrate-1-related 4PS substrate but not the interleukin-2R γ chain is involved in interleukin-13-mediated signal transduction. Blood 86: 4218-4227.
3. Arribas, M., et al. 2003. Essential role of protein kinase C ζ in the impairment of Insulin-induced glucose transport in IRS-2-deficient brown adipocytes. FEBS Lett. 536: 161-166.
4. Pirola, L., et al. 2003. Phosphoinositide 3-kinase-mediated reduction of Insulin receptor substrate-1/2 protein expression via different mechanisms contributes to the Insulin-induced desensitization of its signaling pathways in L6 muscle cells. J. Biol. Chem. 278: 15641-15651.

CHROMOSOMAL LOCATION

Genetic locus: Irs2 (rat) mapping to 16q12.5.

PRODUCT

IRS-2 siRNA (r) 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 IRS-2 shRNA Plasmid (r): sc-155988-SH and IRS-2 shRNA (r) Lentiviral Particles: sc-155988-V as alternate gene silencing products.

For independent verification of IRS-2 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-155988A, sc-155988B and sc-155988C.

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

IRS-2 siRNA (r) is recommended for the inhibition of IRS-2 expression in rat 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

IRS-2 (B-5): sc-390761 is recommended as a control antibody for monitoring of IRS-2 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 IRS-2 gene expression knockdown using RT-PCR Primer: IRS-2 (r)-PR: sc-155988-PR (20 µl, 587 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Nakamura, M., et al. 2015. Preserved Na/HCO_3 cotransporter sensitivity to Insulin may promote hypertension in metabolic syndrome. Kidney Int. 87: 535-542.

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.