

insulin R siRNA (r): sc-63341

BACKGROUND

The Insulin receptor (IR) is a heterodimeric protein complex that has an intracellular β subunit and an extracellular α subunit, which is disulfide-linked to a transmembrane segment. The Insulin ligand binds to the IR and initiates molecular signaling pathways that promote glucose uptake in cells and glycogen synthesis. Insulin binding to IR induces phosphorylation of intracellular tyrosine kinase domains and recruitment of multiple SH2 and SH3 domain-containing intracellular proteins that serve as signaling intermediates for pleiotropic effects of Insulin. The human Insulin receptor gene maps to chromosome 19p13.2 and encodes a 1382 amino acid protein that cleaves apart to form α and β subunits. Type 1 diabetes is an auto-immune condition of the endocrine pancreas that results in destruction of Insulin secreting cells and a progressive loss in Insulin-sensitive glucose uptake by cells. Type 2 diabetes is a condition where cells become resistant to Insulin action.

REFERENCES

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2. Ottensmeyer, F.P., et al. 2000. Mechanism of transmembrane signaling: Insulin binding and the Insulin receptor. *Biochemistry* 39: 12103-12112.
3. Sesti, G. 2000. Insulin receptor variant forms and type 2 diabetes mellitus. *Pharmacogenomics* 1: 49-61.
4. Whitehead, J.P., et al. 2000. Signalling through the Insulin receptor. *Curr. Opin. Cell Biol.* 12: 222-228.
5. Perz, M. and Torlinska, T. 2001. Insulin receptor—structural and functional characteristics. *Med. Sci. Monit.* 7: 169-177.
6. Whitehead, J.P., et al. 2000. Signalling through the Insulin receptor. *Curr. Opin. Cell Biol.* 12: 222-228.
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CHROMOSOMAL LOCATION

Genetic locus: *Insr* (rat) mapping to 12q12.

PRODUCT

insulin R 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Insulin R shRNA Plasmid (r): sc-63341-SH and Insulin R shRNA (r) Lentiviral Particles: sc-63341-V as alternate gene silencing products.

For independent verification of Insulin R (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-63341A, sc-63341B and sc-63341C.

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

insulin R siRNA (r) is recommended for the inhibition of Insulin R 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 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

insulin R β (CT-3): sc-57342 is recommended as a control antibody for monitoring of insulin R 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 Insulin R gene expression knockdown using RT-PCR Primer: Insulin R (r)-PR: sc-63341-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.