SANTA CRUZ BIOTECHNOLOGY, INC.

Insulin siRNA (h): sc-39578

BACKGROUND

Insulin is a secreted peptide hormone that elicits metabolic effects such as increases in glucose uptake and glycogen synthesis leading to a decrease in blood glucose concentration. Insulin is first formed as a precursor molecule, preproInsulin, which is later cleaved to proInsulin and finally to the mature Insulin hormone. Mature Insulin consists of 51 amino acids, contained within an A chain and a B chain that are connected by two disulfide bridges. It increases cell permeability to monosaccharides, amino acids and fatty acids. Insulin is secreted by the pancreas at basal levels in the absence of exogenous stimuli, with secretion increasing in response to glucose. Insulin action is effected by the binding of Insulin to cell-surface receptors on the target cell membrane. Defects of Insulin are the cause of hyperproInsulinemia and of type-II diabetes mellitus.

REFERENCES

1. Kahn, C.R. 1985. The molecular mechanism of Insulin action. Annu. Rev. Med. 36: 429-451.
2. Lammers, R., et al. 1989. Differential signalling potential of Insulin- and IGF-1-receptor cytoplasmic domains. EMBO J. 8: 1369-1375.
3. Hilgert, I., et al. 1991. A monoclonal antibody applicable for determination of C-peptide of human proinsulin by RIA. Hybridoma 10: 379-386.
4. Jorgensen, A.M., et al. 1996. Solution structure of the superactive monomeric des-[Phe(B25)] Insulin and the dimerization of native Insulin. J. Mol. Biol. 257: 684-699.
5. Mackin, R.B. 1998. ProInsulin: recent observations and controversies. Cell. Mol. Life Sci. 54: 696-702.
6. Soria, B., et al. 1998. Cytosolic oscillations and Insulin release in pancreatic islets of Langerhans. Diabetes Metab. 24: 37-40.
7. Walker, M., et al. 2005.Impaired β cell glucose sensitivity and whole-body insulin sensitivity as predictors of hyperglycaemia in non-diabetic subjects. Diabetologia 48: 2470-2476.
8. Polak, J., et al. 2005. Dynamic strength training improves insulin sensitivity and functional balance between adrenergic α 2A and β pathways in subcutaneous adipose tissue of obese subjects. Diabetologia 48: 2631-2640.

CHROMOSOMAL LOCATION

Genetic locus: INS (human) mapping to 11p15.5.

PRODUCT

Insulin 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Insulin shRNA Plasmid (h): sc-39578-SH and Insulin shRNA (h) Lentiviral Particles: sc-39578-V as alternate gene silencing products.

For independent verification of Insulin (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-39578A, sc-39578B and sc-39578C.

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

Insulin siRNA (h) is recommended for the inhibition of Insulin 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 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

Insulin (2D11-H5): sc-8033 is recommended as a control antibody for monitoring of Insulin 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 Insulin gene expression knockdown using RT-PCR Primer: Insulin (h)-PR: sc-39578-PR (20 µl, 359 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.