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Pax-4 siRNA (h): sc-43998

BACKGROUND

Pax-4 (paired box gene 4) protein influences normal differentiation of Insulin-producing β cells and influences normal pancreatic islet development. Pax-4 protein is a transcriptional repressor that binds to a common *cis* element in the glucagon, Insulin and somatostatin promoters. Mouse Pax-4 transcript is present in pancreatic islets, and the islet β cell lines MIN6, β TC, and NIT1. Differentiation of endoderm-derived endocrine pancreas is mediated through Pax-4 and Pax-6. Pax-4 may act as a Pax-6 repressor due to the competition for binding sites and lower transactivation potential of Pax-4. The human Pax-4 gene encodes a deduced 350 amino acid protein that is 80% identical to the deduced mouse Pax-4 protein.

REFERENCES

1. Matsushita, T., et al. 1998. Molecular cloning of mouse paired box containing gene Pax-4 from an islet β cell line and deduced sequence of human Pax-4. *Biochem. Biophys. Res. Commun.* 242: 176-180.
2. Larsson, L.I., et al. 1998. Pax-4 and -6 regulate gastrointestinal endocrine cell development. *Mech. Dev.* 79: 153-159.
3. Kalousová, A., et al. 1999. DNA binding and transactivating properties of the paired and homeobox protein Pax-4. *Biochem. Biophys. Res. Commun.* 259: 510-518.
4. Ritz-Laser, B., et al. 2002. The pancreatic β cell-specific transcription factor Pax-4 inhibits Glucagon gene expression through Pax-6. *Diabetologia* 45: 97-107.
5. Kemp, D.M., et al. 2003. Regulation of Pax-4 paired homeodomain gene by neuron-restrictive silencer factor. *J. Biol. Chem.* 278: 35057-35062.

CHROMOSOMAL LOCATION

Genetic locus: PAX4 (human) mapping to 7q32.1.

PRODUCT

Pax-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 Pax-4 shRNA Plasmid (h): sc-43998-SH and Pax-4 shRNA (h) Lentiviral Particles: sc-43998-V as alternate gene silencing products.

For independent verification of Pax-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-43998A, sc-43998B and sc-43998C.

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.

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

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

Pax (D-7): sc-514352 is recommended as a control antibody for monitoring of Pax-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 Pax-4 gene expression knockdown using RT-PCR Primer: Pax-4 (h)-PR: sc-43998-PR (20 μ l, 497 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.