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Neurogenin 3 siRNA (h): sc-42079

BACKGROUND

The neurogenin family of proteins belongs to the basic helix-loop-helix (bHLH) superfamily and consists of Neurogenin 1, Neurogenin 2 and Neurogenin 3 (also designated ngn3). bHLH members are transcriptional regulators that determine cell fate. Neurogenin 3 is expressed in discrete regions of developing neurons and in the embryonic pancreatic islets of Langerhans. HNF-6 (hepatocyte nuclear factor 6) acts as a positive regulator of Neurogenin 3 by binding to and stimulating the neurogenin gene promoter. Neurogenin 3 is involved in the initial differentiation of the four islets cell types, while a network of transcription factors, including Hlx9, Isl1, Neuro D, Nkx-2.2, Nkx-6.4, Pax-4, Pax-6, PDX-1 and Mash1, are required for final differentiation. Neurogenin 3 acts upstream of Neuro D in a bHLH cascade. Neurogenin 3 activates the expression of Neuro D at the onset of islet cell differentiation through box sequences E1 and E3 in the Neuro D promoter.

REFERENCES

1. Jacquemin, P., et al. 2000. Transcription factor hepatocyte nuclear factor 6 regulates pancreatic endocrine cell differentiation and controls expression of the proendocrine gene ngn3. *Mol. Cell. Biol.* 20: 4445-4454.
2. Gradwohl, G., et al. 2000. Neurogenin3 is required for the development of the four endocrine cell lineages of the pancreas. *Proc. Natl. Acad. Sci. USA* 97: 1607-1611.
3. Schwitzgebel, V.M., et al. 2000. Expression of Neurogenin 3 reveals an islet cell precursor population in the pancreas. *Development* 127: 3533-3542.
4. Jensen, J., et al. 2000. Independent development of pancreatic α - and β -cells from neurogenin3-expressing precursors: a role for the notch pathway in repression of premature differentiation. *Diabetes* 49: 163-176.

CHROMOSOMAL LOCATION

Genetic locus: NEUROG3 (human) mapping to 10q22.1.

PRODUCT

Neurogenin 3 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 Neurogenin 3 shRNA Plasmid (h): sc-42079-SH and Neurogenin 3 shRNA (h) Lentiviral Particles: sc-42079-V as alternate gene silencing products.

For independent verification of Neurogenin 3 (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-42079A, sc-42079B and sc-42079C.

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

Neurogenin 3 siRNA (h) is recommended for the inhibition of Neurogenin 3 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

Neurogenin 3 (E-8): sc-376607 is recommended as a control antibody for monitoring of Neurogenin 3 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 Neurogenin 3 gene expression knockdown using RT-PCR Primer: Neurogenin 3 (h)-PR: sc-42079-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.