SANTA CRUZ BIOTECHNOLOGY, INC.

Phox2b siRNA (m): sc-38765

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The Power to Question

BACKGROUND

Phox2a (also designated Arix1) and Phox2b are closely related, paired-homeodomain transcription factors that are necessary for neuronal differentiation throughout the developing sympathetic, parasympathetic and enteric ganglia. All enteric nervous system cells evolve from the neural crest, and all cells that are undifferentiated initially express Phox2b. The cells that begin to differentiate along a neuronal lineage continue to express Phox2b, and begin to express Phox2a. Phox2b is required for the differentiation of all central and nonperipheral noradrenergic centers in the brain. In contrast, Phox2a controls only the differentiation of the main noradrenergic center of the brain, the locus ceruleus. Both Phox2a and Phox2b are crucial for the regulation of endogenous tyrosine hydroxylase and dopamine-β hydroxylase, which are transiently expressed in neural crest cells. In addition, Phox2 proteins are sufficient to promote sympathetic neuron generation. The gene which encodes Phox2a maps to human chromosome 11q13.4.

REFERENCES

1. Johnson, K.R., et al. 1996. Mapping of the ARIX homeodomain gene to mouse chromosome 7 and human chromosome 11q13. Genomics 33: 527-531.
2. Lo, L., et al. 1999. Specification of neurotransmitter identity by Phox2 proteins in neural crest stem cells. Neuron 22: 693-705.
3. Pattyn, A., et al. 1999. The homeobox gene Phox2b is essential for the development of autonomic neural crest derivatives. Nature 399: 366-370.
4. Young, H.M., et al. 1999. Expression of Ret-, $p75^{NTR}$-, Phox2a-, Phox2b-, and tyrosine hydroxalase-immunoreactivity by undifferentiated neural crest-derived cells and different classes of enteric neurons in the embryonic mouse gut. Dev. Dyn. 216: 137-152.
5. Stanke, M., et al. 1999. The Phox2 homeodomain proteins are sufficient to promote the development of sympathetic neurons. Development 126: 4087-4094.
6. Pattyn, A., et al. 2000. Specification of the central noradrenergic phenotype by the homeobox gene, Phox2b. Mol. Cell. Neurosci. 15: 235-243.

CHROMOSOMAL LOCATION

Genetic locus: Phox2b (mouse) mapping to 5 C3.1.

PRODUCT

Phox2b siRNA (m) 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 Phox2b shRNA Plasmid (m): sc-38765-SH and Phox2b shRNA (m) Lentiviral Particles: sc-38765-V as alternate gene silencing products.

For independent verification of Phox2b (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-38765A, sc-38765B and sc-38765C.

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

Phox2b siRNA (m) is recommended for the inhibition of Phox2b expression in mouse 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

Phox2b (B-11): sc-376997 is recommended as a control antibody for monitoring of Phox2b 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 Phox2b gene expression knockdown using RT-PCR Primer: Phox2b (m)-PR: sc-38765-PR (20 µl). 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.