SANTA CRUZ BIOTECHNOLOGY, INC.

Brn-5 siRNA (m): sc-72665

BACKGROUND

The Brn family of transcription factors are found in a highly restricted subset of neurons and are critical in the early embryonic development of the central nervous system. Brn-1 and Brn-2 are class III POU (Pit-Oct-Unc) domain proteins, Brn-3 is a class IV POU domain protein and Brn-5 is a class VI POU domain protein. Brn-5 (Brain-5), also known as POU6F1, MPOU or TCFB1, is a widely expressed protein but during embryogenesis is exclusively found in the developing brain and spinal cord. As is characteristic of Brn family members, Brn-5 contains two DNA-binding domains, namely the POU-specific domain and the POU homeodomain, which each contain an HTH (helix-turn-helix) motif. Brn-5 binds to CRH (corticotrophin-releasing hormone) elements with high affinity and is capable of both enhancing Prolactin gene expression and activating Pit-1 expression.

REFERENCES

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2. Gruber, C.A., et al. 1997. POU domain factors of the Brn-3 class recognize functional DNA elements which are distinctive, symmetrical, and highly conserved in evolution. Mol. Cell. Biol. 17: 2391-2400.
3. Rhee, J.M., et al. 1998. Highly cooperative homodimerization is a conserved property of neural POU proteins. J. Biol. Chem. 273: 34196-34205.
4. Cui, H., et al. 1998. Expression of the POU transcription factor Brn-5 is an early event in the terminal differentiation of CNS neurons. J. Neurosci. Res. 52: 625-632.
5. Donahue, L.M., et al. 1998. POU domain genes are differentially expressed in the early stages after lineage commitment of the PNS-derived stem cell line, RT4-AC. Brain Res. Dev. Brain Res. 106: 1-12.
6. Cui, H., et al. 1998. Potassium chloride inhibits proliferation of cerebellar granule neuron progenitors. Brain Res. Dev. Brain Res. 106: 129-135.
7. Wu, R., et al. 2001. The POU gene Brn-5 is induced by neuregulin and is restricted to myelinating Schwann cells. Mol. Cell. Neurosci. 17: 683-695.
8. Pereira, J.H., et al. 2008. Crystallization and preliminary X-ray analysis of human Brn-5 transcription factor in complex with DNA. Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 64: 175-178.

CHROMOSOMAL LOCATION

Genetic locus: Pou6f1 (mouse) mapping to 15 F1.

PRODUCT

Brn-5 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 Brn-5 shRNA Plasmid (m): sc-72665-SH and Brn-5 shRNA (m) Lentiviral Particles: sc-72665-V as alternate gene silencing products.

For independent verification of Brn-5 (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-72665A, sc-72665B and sc-72665C.

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

Brn-5 siRNA (m) is recommended for the inhibition of Brn-5 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

Brn-5 (E-30): sc-81982 is recommended as a control antibody for monitoring of Brn-5 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 Brn-5 gene expression knockdown using RT-PCR Primer: Brn-5 (m)-PR: sc-72665-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.