SANTA CRUZ BIOTECHNOLOGY, INC.

Neuro D siRNA (m): sc-36034

BACKGROUND

The basic helix-loop-helix (bHLH) proteins are transcription factors that are required for several aspects of development, including cell type determination, terminal differentiation and sex determination. The HLH domain is required for dimerization, while the basic region makes specific contacts with DNA. Members of the myogenic determination family, MyoD, Myf-5, myogenin and Mrf-4, all have bHLH domains. These proteins heterodimerize with members of the E protein family and initiate myogenesis. Neuro D has been identified as a bHLH transcription factor functioning in neurogenic differentiation. Neuro D is expressed transiently in a subset of neurons in the central and peripheral nervous systems at the time of their terminal differentiation into mature neurons. Moreover, ectopic expression of Neuro D in *Xenopus* embryos induces premature differentiation of neuronal precursors and Neuro D can convert presumptive epidermal cells into neurons.

REFERENCES

1. Ferre-D'Amare, A.R., et al. 1993. Recognition by Max of its cognate DNA through a dimeric b/HLH/z domain. Nature 363: 38-45.
2. Ellenberger, T., et al. 1994. Crystal structure of transcription factor E47: E-box recognition by a basic region helix-loop-helix dimer. Genes Dev. 8: 970-980.
3. Ma, P.C., et al. 1994. Crystal structure of MyoD bHLH domain-DNA complex: perspectives on DNA recognition and implications for transcriptional activation. Cell 77: 451-459.
4. Lee, J.E., et al. 1995. Conversion of *Xenopus* ectoderm into neurons by Neuro D, a basic helix-loop-helix protein. Science 268: 836-844.
5. Baudier, J., et al. 1995. Interactions of myogenic bHLH transcription factors with calcium-binding calmodulin and S100a ($\alpha\alpha$) proteins. Biochemistry 34: 7834-7846.
6. Zhang, W., et al. 1995. Inactivation of the myogenic bHLH gene MRF4 results in up-regulation of myogenin and rib anomalies. Genes Dev. 9: 1388-1399.

CHROMOSOMAL LOCATION

Genetic locus: Neurod1 (mouse) mapping to 2 C3.

PRODUCT

Neuro D 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 Neuro D shRNA Plasmid (m): sc-36034-SH and Neuro D shRNA (m) Lentiviral Particles: sc-36034-V as alternate gene silencing products.

For independent verification of Neuro D (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-36034A, sc-36034B and sc-36034C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

Neuro D siRNA (m) is recommended for the inhibition of Neuro D 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

Neuro D (G-12): sc-398891 is recommended as a control antibody for monitoring of Neuro D 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 Neuro D gene expression knockdown using RT-PCR Primer: Neuro D (m)-PR: sc-36034-PR (20 µl, 606 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.