

EN-1 siRNA (m): sc-45653

BACKGROUND

The engrailed-1 gene, EN1, a murine homolog of the *Drosophila* homeobox gene engrailed (EN), is required for midbrain and cerebellum development and dorsal/ventral patterning of the limbs as well as apical ectodermal ridge formation. In *Drosophila*, the EN gene plays an important role during development in segmentation, where it is required for the formation of posterior compartments. Human EN-1 and EN-2 are homeodomain-containing proteins and have been implicated in the control of pattern formation during development of the central nervous system. Different mutations in the mouse homologs, EN-1 and EN-2, produce different developmental defects that frequently are lethal. EN-1 is highly expressed by essentially all dopaminergic neurons in the substantia nigra and ventral tegmentum. EN-1 and EN-2 regulate expression of α -synuclein, a gene that is genetically linked to Parkinson's disease.

REFERENCES

1. Kohler, A., et al. 1993. Regional assignment of the human homeobox-containing gene EN1 to chromosome 2q13-q21. *Genomics* 15: 233-235.
2. Hanks, M.C., et al. 1998. *Drosophila* engrailed can substitute for mouse Engrailed-1 function in mid-hindbrain, but not limb development. *Development* 125: 4521-4530.
3. Ohuchi, H., et al. 1999. FGF-10 can induce FGF-8 expression concomitantly with EN-1 and R-fng expression in chick limb ectoderm, independent of its dorsoventral specification. *Dev. Growth Differ.* 41: 665-673.
4. Gemel, J., et al. 1999. Fibroblast growth factor-8 expression is regulated by intronic engrailed and Pbx 1-binding sites. *J. Biol. Chem.* 274: 6020-6026.
5. Simon, H.H., et al. 2001. Fate of midbrain dopaminergic neurons controlled by the engrailed genes. *J. Neurosci.* 21: 3126-3134.
6. Simon, H.H., et al. 2004. Midbrain dopaminergic neurons: control of their cell fate by the engrailed transcription factors. *Cell Tissue Res.* 318: 53-61.
7. Alberi, L., et al. 2004. Engrailed genes are cell-autonomously required to prevent apoptosis in mesencephalic dopaminergic neurons. *Development* 131: 3229-3236.
8. LocusLink Report (LocusID: 2019). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: En1 (mouse) mapping to 1 E2.3.

PRODUCT

EN-1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see EN-1 shRNA Plasmid (m): sc-45653-SH and EN-1 shRNA (m) Lentiviral Particles: sc-45653-V as alternate gene silencing products.

For independent verification of EN-1 (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-45653A, sc-45653B and sc-45653C.

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

EN-1 shRNA (m) Lentiviral Particles is recommended for the inhibition of EN-1 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 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

EN-1 (E-12): sc-398534 is recommended as a control antibody for monitoring of EN-1 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 EN-1 gene expression knockdown using RT-PCR Primer: EN-1 (m)-PR: sc-45653-PR (20 μ l, 443 bp). 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.