



## EN-2 siRNA (m): sc-72138

### BACKGROUND

The engrailed-2 gene, EN2, 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  $\alpha$ -synuclein, a gene that is genetically linked to Parkinson's disease. During early brain development mouse EN-2 is expressed in a broad band across most of the mid-hindbrain region. EN-2 is also expressed in mouse myoblasts and has been associated with cerebellar hypoplasia.

### REFERENCES

1. Goldfarb, A.N., et al. 1992. T cell acute lymphoblastic leukemia—the associated gene SCL/TAL codes for a 42-Kd nuclear phosphoprotein. *Blood* 80: 2858-2866.
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. FGF10 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. Li Song, D., et al. 2000. Two Pax-2/5/8-binding sites in engrailed-2 are required for proper initiation of endogenous mid-hindbrain expression. *Mech. Dev.* 90: 155-165.

### CHROMOSOMAL LOCATION

Genetic locus: En2 (mouse) mapping to 5 B1.

### PRODUCT

EN-2 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see EN-2 shRNA Plasmid (m): sc-72138-SH and EN-2 shRNA (m) Lentiviral Particles: sc-72138-V as alternate gene silencing products.

For independent verification of EN-2 (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-72138A, sc-72138B and sc-72138C.

### PROTOCOLS

See our web site at [www.scbt.com](http://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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

EN-2 siRNA (m) is recommended for the inhibition of EN-2 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  $\mu$ M in 66  $\mu$ 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.

### RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor EN-2 gene expression knockdown using RT-PCR Primer: EN-2 (m)-PR: sc-72138-PR (20  $\mu$ l). 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.