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# EN-1 shRNA (m) Lentiviral Particles: sc-45653-V

## 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  $\alpha$ -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.

## CHROMOSOMAL LOCATION

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

## PRODUCT

EN-1 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see EN-1 siRNA (m): sc-45653 and EN-1 shRNA Plasmid (m): sc-45653-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ\text{C}$ . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ\text{C}$  for up to one week. Avoid repeated freeze thaw cycles.

## APPLICATIONS

EN-1 shRNA (m) Lentiviral Particles is recommended for the inhibition of EN-1 expression in mouse cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgM-HRP: sc-2064 (dilution range: 1:500-1:5,000), TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgM-FITC: sc-2082 (dilution range: 1:100-1:400) or goat anti-mouse IgM-TR: sc-2983 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## 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  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ\text{C}$  and the extension temperature should be  $68-72^\circ\text{C}$ .

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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