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### SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

[mail@szabo-scandic.com](mailto:mail@szabo-scandic.com)

[www.szabo-scandic.com](http://www.szabo-scandic.com)

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

# EN-2 shRNA (h) Lentiviral Particles: sc-45658-V

## BACKGROUND

The engrailed-2 gene, EN-2, 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

- 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.
- 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.
- 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.
- 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.
- Li Song, D. and Joyner, A.L. 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 (human) mapping to 7q36.3.

## PRODUCT

EN-2 shRNA (h) 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-2 siRNA (h): sc-45658 and EN-2 shRNA Plasmid (h): sc-45658-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-2 shRNA (h) Lentiviral Particles is recommended for the inhibition of EN-2 expression in human 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-2 (H-95): sc-66877 is recommended as a control antibody for monitoring of EN-2 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (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-2 gene expression knockdown using RT-PCR Primer: EN-2 (h)-PR: sc-45658-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.

## PROTOCOLS

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