SANTA CRUZ BIOTECHNOLOGY, INC.

EN-2 siRNA (h): sc-45658

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The Power to Question

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 forma-tion. 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. During early brain development mouse EN-2 is expressed in a broad band across most of themid-hindbrain region. EN-2 is also expressed in mouse myoblasts and has been assiciated with cerebellar hypoplasia.

REFERENCES

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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. 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.
6. Simon, H.H., et al. 2001. Fate of midbrain dopaminergic neurons controlled by the engrailed genes. J. Neurosci. 21: 3126-3134.

CHROMOSOMAL LOCATION

Genetic locus: EN2 (human) mapping to 7q36.3.

PRODUCT

EN-2 siRNA (h) 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 EN-2 shRNA Plasmid (h): sc-45658-SH and EN-2 shRNA (h) Lentiviral Particles: sc-45658-V as alternate gene silencing products.

For independent verification of EN-2 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45658A, sc-45658B and sc-45658C.

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

EN-2 shRNA (h) Lentiviral Particles is recommended for the inhibition of EN-2 expression in human 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

EN-2 (1E1): sc-293311 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 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 EN-2 gene expression knockdown using RT-PCR Primer: EN-2 (h)-PR: sc-45658-PR (20 µl). 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.