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OTX1/2 shRNA (h) Lentiviral Particles: sc-43985-V

BACKGROUND

Transcription factors OTX1 and OTX2, two murine homologs of the *Drosophila* orthodenticle (OTD), show a limited amino acid sequence divergence. OTX1 and OTX2 play an important role during early and later events required for proper brain development in that they are involved in the processes of induction, specification and regionalization of the brain. OTX1 is involved in corticogenesis, sensory organ development and pituitary functions, while OTX2 is necessary earlier in development, for the correct anterior neural plate specification and organization of the primitive streak. OTX2 is also required in the early specification of the neuroectoderm, which is destined to become the fore-midbrain, and both OTX1 and OTX2 co-operate in patterning the developing brain through a dosage-dependent mechanism. A molecular mechanism depending on a precise threshold of OTX proteins is necessary for the correct positioning of the isthmic region and for anterior brain patterning. The genes which encode OTX1 and OTX2 map to human chromosomes 2p15 and 14q22.3, respectively.

REFERENCES

1. Kastury, K., et al. 1994. Chromosome locations of human EMX and OTX genes. *Genomics* 22: 41-45.
2. Acampora, D., et al. 1999. Differential transcriptional control as the major molecular event in generating OTX1^{-/-} and OTX2^{-/-} divergent phenotypes. *Development* 126: 1417-1426.
3. Acampora, D., et al. 1999. OTX genes in corticogenesis and brain development. *Cereb. Cortex* 9: 533-542.
4. Acampora, D. et al. 1999. The TINS Lecture. Understanding the roles of OTX1 and OTX2 in the control of brain morphogenesis. *Trends Neurosci.* 22: 116-122.
5. Acampora, D., et al. Gulisano, M. and Simeone, A. 1999. OTX genes and the genetic control of brain morphogenesis. *Mol. Cell. Neurosci.* 13: 1-8.
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CHROMOSOMAL LOCATION

Genetic locus: OTX1 (human) mapping to 2p15, OTX2 (human) mapping to 14q22.3.

PRODUCT

OTX1/2 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 4 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10⁶ 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 OTX1/2 siRNA (h): sc-43985 and OTX1/2 shRNA Plasmid (h): sc-43985-SH as alternate gene silencing products.

STORAGE

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

APPLICATIONS

OTX1/2 shRNA (h) Lentiviral Particles is recommended for the inhibition of OTX1 and OTX2 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

OTX1/2 (N-15): sc-11026 is recommended as a control antibody for monitoring of OTX1 and OTX2 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor OTX1 and OTX2 gene expression knockdown using RT-PCR Primer: OTX1/2 (h)-PR: sc-43985-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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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