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ODC shRNA (m) Lentiviral Particles: sc-44573-V

BACKGROUND

Ornithine decarboxylase (ODC) is an enzyme that performs the first step in polyamine biosynthesis by converting ornithine to putrescine and CO₂. ODC plays an important role in diverse biological processes, including cell growth, differentiation, transformation and apoptosis. The Sp1, c-Myc and c-Fos genes function as transactivators and ZBP-89 as a transrepressor of the ODC promoter. Overexpression of ODC gene plays important roles in cell proliferation and the development of cancer. High levels of protein binding in the ODC promoter are implicated to the elevated constitutive expression of this gene. Elevated polyamine levels lead to downregulation of ODC activity by enhancing the translation of antizyme mRNA, resulting in subsequent binding of antizyme to ODC monomers to target ODC for proteolysis by the 26S Proteasome. DFMO (DL- α -difluoromethylornithine) is an irreversible inhibitor of ODC, which can induce apoptosis and inhibits cell growth. ODC is also associated with angiogenesis, and ODC-overexpressing cells exhibit suppressed expression of Type XVIII Collagen and endostatin, suggesting that overexpression of ODC facilitates endothelial proliferation by suppressing endostatin expression. The ODC gene maps to human chromosome 2p25.

REFERENCES

1. Tabor, C.W., et al. 1984. Polyamines. *Annu. Rev. Biochem.* 53: 749-790.
2. Yang-Feng, T.L., et al. 1987. Ribonucleotide reductase M2 subunit sequences mapped to four different chromosomal sites in humans and mice: functional locus identified by its amplification in hydroxyurea-resistant cell lines. *Genomics* 1: 77-86.
3. Pegg, A.E. 1988. Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy. *Cancer Res.* 48: 759-774.
4. Heby, O., et al. 1990. Molecular genetics of polyamine synthesis in eucaryotic cells. *Trends Biochem. Sci.* 15: 153-158.
5. Moshier, J.A., et al. 1992. Multiple promoter elements govern expression of the human ornithine decarboxylase gene in colon carcinoma cells. *Nucleic Acids Res.* 20: 2581-2590.

CHROMOSOMAL LOCATION

Genetic locus: Odc1 (mouse) mapping to 12 A1.1.

PRODUCT

ODC 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 μ l 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 ODC siRNA (m): sc-44573 and ODC shRNA Plasmid (m): sc-44573-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

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

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l 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

ODC (E-6): sc-398116 is recommended as a control antibody for monitoring of ODC 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 IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ODC gene expression knockdown using RT-PCR Primer: ODC (m)-PR: sc-44573-PR (20 μ l, 590 bp). 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.

PROTOCOLS

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