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Alpha 4 shRNA (h) Lentiviral Particles: sc-44648-V

BACKGROUND

Alpha 4 is a cytoplasmic protein which associates with surface IgM-receptor and may help regulate signal transduction. Alpha 4 regulates the catalytic activity of type 2A-related serine/threonine phosphatases (PP2A) and interacts with MID1, the product of the gene mutated in X-linked Opitz GBBB syndrome. PP2Ac accumulation is caused by an impairment of E3 ubiquitin ligase activity of the MID1 protein which normally targets PP2Ac for degradation through binding to its Alpha 4 regulatory subunit. Patients with Opitz GBBB syndrome suffer from a variable array of developmental defects including craniofacial, cardiac and genital anomalies. Alpha 4 is present at highest levels in heart, skeletal muscle and pancreas, and is a member of the IGBP1/TAP42 family.

REFERENCES

1. Trockenbacher, A., et al. 2001. MID1, mutated in Opitz syndrome, encodes an ubiquitin ligase that targets phosphatase 2A for degradation. *Nat. Genet.* 29: 287-294.
2. Liu, J., et al. 2001. Phosphorylation and microtubule association of the Opitz syndrome protein mid-1 is regulated by protein phosphatase 2A via binding to the regulatory subunit Alpha 4. *Proc. Natl. Acad. Sci. USA* 98: 6650-6655.
3. Short, K.M., et al. 2002. MID1 and MID2 homo- and heterodimerise to tether the rapamycin-sensitive PP2A regulatory subunit, alpha 4, to microtubules: implications for the clinical variability of X-linked Opitz GBBB syndrome and other developmental disorders. *BMC Cell Biol.* 3: 1.
4. Everett, A.D., et al. 2002. Developmental expression of Alpha4 protein phosphatase regulatory subunit in tissues affected by Opitz syndrome. *Dev. Dyn.* 224: 461-464.
5. Graham, J.M., Jr., et al. 2003. A new X-linked syndrome with agenesis of the corpus callosum, mental retardation, coloboma, micrognathia, and a mutation in the Alpha 4 gene at Xq13. *Amer. J. Med. Genet. A* 123: 37-44.
6. SWISS-PROT/TrEMBL (P78318). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: IGBP1 (human) mapping to Xq13.1.

PRODUCT

Alpha 4 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 μ l frozen stock containing 1.0×10^6 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 Alpha 4 siRNA (h): sc-44648 and Alpha 4 shRNA Plasmid (h): sc-44648-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

Alpha 4 shRNA (h) Lentiviral Particles is recommended for the inhibition of Alpha 4 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

Alpha 4 (B-5): sc-373719 is recommended as a control antibody for monitoring of Alpha 4 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 Alpha 4 gene expression knockdown using RT-PCR Primer: Alpha 4 (h)-PR: sc-44648-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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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