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Alpha 4 siRNA (h): sc-44648

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 MID1 is regulated by protein phosphatase 2A via binding to the regulatory subunit Alpha 4. *Proc. Natl. Acad. Sci. USA* 98: 6650-6655.
3. Everett, A.D., et al. 2002. Developmental expression of Alpha 4 protein phosphatase regulatory subunit in tissues affected by Opitz syndrome. *Dev. Dyn.* 224: 461-464.
4. 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.
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. *Am. J. Med. Genet. A* 123A: 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 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Alpha 4 shRNA Plasmid (h): sc-44648-SH and Alpha 4 shRNA (h) Lentiviral Particles: sc-44648-V as alternate gene silencing products.

For independent verification of Alpha 4 (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-44648A, sc-44648B and sc-44648C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Alpha 4 siRNA (h) is recommended for the inhibition of Alpha 4 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 μ M in 66 μ l. 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

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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 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° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.