

ALDH4A1 siRNA (m): sc-72479

BACKGROUND

Aldehyde dehydrogenases (ALDHs) mediate NADP⁺-dependent oxidation of aldehydes into acids during detoxification of alcohol-derived acetaldehyde, lipid peroxidation and metabolism of corticosteroids, biogenic amines and neurotransmitters. ALDH4A1 (aldehyde dehydrogenase 4 family member A1), also known as P5CD (Δ^1 -pyrroline-5-carboxylate dehydrogenase), P5CDh, P5CDhL, P5CDhS or ALDH4, is a major enzyme involved in the proline degradation pathway. Localizing to the mitochondrial matrix, ALDH4A1 catalyzes the conversion of Δ^1 -pyrroline-5-carboxylate (P5C) to glutamate. A mutation in the gene encoding ALDH4A1 results in HPII (hyperprolinemia type II), a disease characterized by an excess of P5C and proline that is associated with mental retardation and seizures.

REFERENCES

1. Goodman, S.I., Mace, J.W., Miles, B.S., Teng, C.C. and Brown, S.B. 1974. Defective hydroxyproline metabolism in type II hyperprolinemia. *Biochem. Med.* 10: 329-336.
2. Flynn, M.P., Martin, M.C., Moore, P.T., Stafford, J.A., Fleming, G.A. and Phang, J.M. 1989. Type II hyperprolinaemia in a pedigree of Irish travellers (nomads). *Arch. Dis. Child.* 64: 1699-1707.
3. Yoshiba, Y., Kiyosue, T., Nakashima, K., Yamaguchi-Shinozaki, K. and Shinozaki, K. 1997. Regulation of levels of proline as an osmolyte in plants under water stress. *Plant Cell Physiol.* 38: 1095-1102.
4. Geraghty, M.T., Vaughn, D., Nicholson, A.J., Lin, W.W., Jimenez-Sanchez, G., Obie, C., Flynn, M.P., Valle, D. and Hu, C.A. 1998. Mutations in the Δ^1 -pyrroline 5-carboxylate dehydrogenase gene cause type II hyperprolinemia. *Hum. Mol. Genet.* 7: 1411-1415.
5. Vasiliou, V., Bairoch, A., Tipton, K.F. and Nebert, D.W. 1999. Eukaryotic aldehyde dehydrogenase (ALDH) genes: human polymorphisms, and recommended nomenclature based on divergent evolution and chromosomal mapping. *Pharmacogenetics* 9: 421-434.

CHROMOSOMAL LOCATION

Genetic locus: *Aldh4a1* (mouse) mapping to 4 D3.

PRODUCT

ALDH4A1 siRNA (m) 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 ALDH4A1 shRNA Plasmid (m): sc-72479-SH and ALDH4A1 shRNA (m) Lentiviral Particles: sc-72479-V as alternate gene silencing products.

For independent verification of ALDH4A1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-72479A, sc-72479B and sc-72479C.

PROTOCOLS

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

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

ALDH4A1 siRNA (m) is recommended for the inhibition of ALDH4A1 expression in mouse 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

ALDH4A1 (F-1): sc-398911 is recommended as a control antibody for monitoring of ALDH4A1 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 ALDH4A1 gene expression knockdown using RT-PCR Primer: ALDH4A1 (m)-PR: sc-72479-PR (20 μ l). 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.