

ADH5 siRNA (h): sc-105044

BACKGROUND

The alcohol dehydrogenase family of proteins metabolize a wide variety of substrates, including retinol, hydroxysteroids, ethanol, aliphatic alcohols and lipid peroxidation products. ADH5 (alcohol dehydrogenase 5 (class III)), also known as FDH (formaldehyde dehydrogenase), ADHX, ADH-3 or GSNOR, is a 374 amino acid cytoplasmic protein that belongs to the class III subfamily of alcohol dehydrogenases. Expressed ubiquitously, ADH5 uses iron as a cofactor to catalytically oxidize both long-chain primary alcohols and S-hydroxymethyl-glutathione, a product formed spontaneously between formaldehyde and glutathione. ADH5 exists as a homodimer and, via its ability to oxidize S-hydroxymethyl-glutathione and thus eliminate formaldehyde, functions as an important component of cellular metabolism. Genetic variations in the gene encoding ADH5 may affect drug and alcohol dependence in humans.

REFERENCES

1. Kaiser, R., et al. 1988. Class III human liver alcohol dehydrogenase: a novel structural type equidistantly related to the class I and class II enzymes. *Biochemistry* 27: 1132-1140.
2. Giri, P.R., et al. 1989. Cloning and comparative mapping of a human class III (χ) alcohol dehydrogenase cDNA. *Biochem. Biophys. Res. Commun.* 164: 453-460.
3. Hur, M.W. and Edenberg, H.J. 1992. Cloning and characterization of the ADH5 gene encoding human alcohol dehydrogenase 5, formaldehyde dehydrogenase. *Gene* 121: 305-311.
4. Holmquist, B., et al. 1993. Role of Arginine 115 in fatty acid activation and formaldehyde dehydrogenase activity of human class III alcohol dehydrogenase. *Biochemistry* 32: 5139-5144.
5. Engeland, K., et al. 1993. Mutation of Arg 115 of human class III alcohol dehydrogenase: a binding site required for formaldehyde dehydrogenase activity and fatty acid activation. *Proc. Natl. Acad. Sci. USA* 90: 2491-2494.
6. Yang, Z.N., et al. 1997. Structure of human chi chi alcohol dehydrogenase: a glutathione-dependent formaldehyde dehydrogenase. *J. Mol. Biol.* 265: 330-343.

CHROMOSOMAL LOCATION

Genetic locus: ADH5 (human) mapping to 4q23.

PRODUCT

ADH5 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 ADH5 shRNA Plasmid (h): sc-105044-SH and ADH5 shRNA (h) Lentiviral Particles: sc-105044-V as alternate gene silencing products.

For independent verification of ADH5 (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-105044A, sc-105044B and sc-105044C.

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

ADH5 siRNA (h) is recommended for the inhibition of ADH5 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

ADH5 (B-1): sc-518202 is recommended as a control antibody for monitoring of ADH5 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 ADH5 gene expression knockdown using RT-PCR Primer: ADH5 (h)-PR: sc-105044-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.

PROTOCOLS

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