

ADH8 siRNA (h): sc-77632

BACKGROUND

The alcohol dehydrogenase family of proteins metabolize a wide variety of substrates, including retinol, hydroxysteroids, ethanol, aliphatic alcohols and lipid peroxidation products. ADH8, also known as ADHFE1 (alcohol dehydrogenase, iron containing, 1) or HOT, is a 467 amino acid protein that belongs to the iron-containing alcohol dehydrogenase family and localizes to the mitochondrion. Expressed specifically in adult liver, ADH8 functions to catalyze the cofactor-independent oxidation of γ -hydroxybutyrate to succinic semialdehyde, a reaction that is coupled to the reduction of 2-ketoglutarate to D-2-hydroxyglutarate and occurs at an optimal pH of 7.5. Succinic semialdehyde can then be converted to succinic acid which is used for energy production in the Krebs cycle. Four isoforms of ADH8 exist due to alternative splicing events.

REFERENCES

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- Rosell, A., et al. 2003. Crystal structure of the vertebrate NADP(H)-dependent alcohol dehydrogenase (ADH8). *J. Mol. Biol.* 330: 75-85.
- Reimers, M.J., et al. 2004. Two zebrafish alcohol dehydrogenases share common ancestry with mammalian class I, II, IV, and V alcohol dehydrogenase genes but have distinct functional characteristics. *J. Biol. Chem.* 279: 38303-38312.
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CHROMOSOMAL LOCATION

Genetic locus: ADHFE1 (human) mapping to 8q13.1.

PRODUCT

ADH8 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 ADH8 shRNA Plasmid (h): sc-77632-SH and ADH8 shRNA (h) Lentiviral Particles: sc-77632-V as alternate gene silencing products.

For independent verification of ADH8 (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-77632A, sc-77632B and sc-77632C.

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

ADH8 siRNA (h) is recommended for the inhibition of ADH8 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

ADH8 (F-9): sc-390387 is recommended as a control antibody for monitoring of ADH8 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 ADH8 gene expression knockdown using RT-PCR Primer: ADH8 (h)-PR: sc-77632-PR (20 μ l, 553 bp). 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.