SANTA CRUZ BIOTECHNOLOGY, INC.

HIBADH siRNA (m): sc-145957

BACKGROUND

HIBADH (3-hydroxyisobutyrate dehydrogenase) is a 336 amino acid mitochondrial enzyme that catalyzes the NAD^+-dependent, reversible oxidization of 3-hydroxyisobutyrate to methylmalonate semialdehyde, an intermediate of valine catabolism. The enzyme functions as a homodimer between a pH of 7.0 and 10.0, with optimal activity between 8.8 and 9.0. It was previously hypothesized that defects in the gene encoding HIBADH may be the cause of 3-hydroxyisobutyric aciduria, a rare disorder that is characterized by a variety of clinical manifestations such as neurodevelopmental problems and dysmorphic features. However, it was shown that HIBADH activity was equal in patients with 3-hydroxyisobutyric aciduria as compared with controls.

REFERENCES

1. Rougraff, P.M., et al. 1989. Cloning and sequence analysis of a cDNA for 3-hydroxyisobutyrate dehydrogenase. Evidence for its evolutionary relationship to other pyridine nucleotide-dependent dehydrogenases. J. Biol. Chem. 264: 5899-5903.
2. Lokanath, N.K., et al. 2003. Crystallization and preliminary X-ray crystallographic studies of NADP-dependent 3-hydroxyisobutyrate dehydrogenase from *Thermus thermophilus* HB8. Acta Crystallogr. D Biol. Crystallogr. 59: 2294-2296.
3. Lehoczky, J.A., et al. 2004. Conserved expression domains for genes upstream and within the HoxA and HoxD clusters suggests a long-range enhancer existed before cluster duplication. Evol. Dev. 6: 423-430.
4. Online Mendelian Inheritance in Man, OMIM™. 2004. Johns Hopkins University, Baltimore, MD. MIM Number: 608475. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Lokanath, N.K., et al. 2005. Crystal structure of novel NADP-dependent 3-hydroxyisobutyrate dehydrogenase from *Thermus thermophilus* HB8. J. Mol. Biol. 352: 905-917.
6. Loupatty, F.J., et al. 2006. Clinical, biochemical, and molecular findings in three patients with 3-hydroxyisobutyric aciduria. Mol. Genet. Metab. 87: 243-248.

CHROMOSOMAL LOCATION

Genetic locus: Hibadh (mouse) mapping to 6 B3.

PRODUCT

HIBADH 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HIBADH shRNA Plasmid (m): sc-145957-SH and HIBADH shRNA (m) Lentiviral Particles: sc-145957-V as alternate gene silencing products.

For independent verification of HIBADH (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-145957A, sc-145957B and sc-145957C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

HIBADH siRNA (m) is recommended for the inhibition of HIBADH 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 µM in 66 µl. 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

HIBADH (D-11): sc-398288 is recommended as a control antibody for monitoring of HIBADH 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 HIBADH gene expression knockdown using RT-PCR Primer: HIBADH (m)-PR: sc-145957-PR (20 µl). 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.