

DHS siRNA (h): sc-60535

BACKGROUND

Deoxyhypusine synthase (DHS) is crucial for the post-translational formation of hypusine, a modification of a specific lysine residue in eukaryotic initiation factor 5A (eIF-5A). Hypusine is formed by posttranslational modifications involving two enzymatic steps catalyzed by DHS and deoxyhypusine hydroxylase (DOHH). eIF-5A is essential for eukaryotic cell proliferation. Deoxyhypusine synthase, which belongs to the deoxyhypusine synthase family of proteins, is important for the first step in the hypusine biosynthesis pathway. It acts as a catalyst for the NAD-dependent oxidative cleavage of spermidine and the ensuing transfer of the butylamine moiety of spermidine to the eIF-5A protein, to create the intermediate deoxyhypusine residue.

REFERENCES

- Huang, J.K., et al. 2004. Molecular cloning of bovine eIF-5A and deoxyhypusine synthase cDNA. *DNA Seq.* 15: 26-32.
- Xu, A., et al. 2004. Identification of mRNA that binds to eukaryotic initiation factor 5A by affinity co-purification and differential display. *Biochem. J.* 384: 585-590.
- Sommer, M.N., et al. 2004. Screening assay for the identification of deoxyhypusine synthase inhibitors. *J. Biomol. Screen.* 9: 434-438.
- Molitor, I.M., et al. 2004. Translation initiation factor eIF-5A from *Plasmodium falciparum*. *Mol. Biochem. Parasitol.* 137: 65-74.
- Umland, T.C., et al. 2004. A new crystal structure of deoxyhypusine synthase reveals the configuration of the active enzyme and of an enzyme.NAD.inhibitor ternary complex. *J. Biol. Chem.* 279: 28697-28705.
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- Hauber, I., et al. 2005. Identification of cellular deoxyhypusine synthase as a novel target for antiretroviral therapy. *J. Clin. Invest.* 115: 76-85.
- Nishimura, K., et al. 2005. Independent roles of eIF-5A and polyamines in cell proliferation. *Biochem. J.* 385: 779-785.

CHROMOSOMAL LOCATION

Genetic locus: DHPS (human) mapping to 19p13.2.

PRODUCT

DHS 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 DHS shRNA Plasmid (h): sc-60535-SH and DHS shRNA (h) Lentiviral Particles: sc-60535-V as alternate gene silencing products.

For independent verification of DHS (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-60535A, sc-60535B and sc-60535C.

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

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

DHS (A-10): sc-365077 is recommended as a control antibody for monitoring of DHS 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 DHS gene expression knockdown using RT-PCR Primer: DHS (h)-PR: sc-60535-PR (20 μ l, 483 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.