SANTA CRUZ BIOTECHNOLOGY, INC.

DOHH siRNA (m): sc-62223

BACKGROUND

DOHH (deoxyhypusine hydroxylase/monooxygenase), also known as HLRC1 (HEAT-like (PBS lyase) repeat containing 1), is a metalloenzyme involved in hypusine synthesis. It contains eight tandem HEAT-repeats, four at the N-terminus and four at the C-terminus. DOHH is an important player in mediating the posttranslational modifications of eIF5a to form hypusine. The first step of this reaction is catalyzed by DHS (deoxyhypusine synthase), which is responsible for transferring the aminobutyl moiety of spermidine to a lysine residue of eIF5a to form a deoxyhypusine-containing eIF5a intermediate. DOHH catalyzes the second step, hydroxylating the intermediate to form the hypusine residue thereby activating eIF5a. DHS, DOHH and eIF5a are evolutionarily conserved proteins that are essential for cell proliferation. Inhibition of DOHH can result in cell cycle arrest at the G_1/S boundary. This suggests a potential use of DOHH inhibitors in antitumor therapy.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 611262. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
2. Brochier, C., et al. 2004. Horizontal gene transfer and archaeal origin of deoxyhypusine synthase homologous genes in bacteria. Gene 330: 169-176.
3. Sommer, M.N., et al. 2004. Screening assay for the identification of deoxyhypusine synthase inhibitors. J. Biomol. Screen 9: 434-438.
4. Park, M.H. 2006. The post-translational synthesis of a polyamine-derived amino acid, hypusine, in the eukaryotic translation initiation factor 5A (eIF5A). J. Biochem. 139: 161-169.
5. Park, J.H., et al. 2006. Molecular cloning, expression, and structural prediction of deoxyhypusine hydroxylase: a HEAT-repeat-containing metalloenzyme. Proc. Natl. Acad. Sci. USA 103: 51-56.
6. Jao, D.L., et al. 2006. Tandem affinity purification revealed the hypusine-dependent binding of eukaryotic initiation factor 5A to the translating 80S ribosomal complex. J. Cell. Biochem. 97: 583-598.

CHROMOSOMAL LOCATION

Genetic locus: Dohh (mouse) mapping to 10 C1.

PRODUCT

DOHH 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 DOHH shRNA Plasmid (m): sc-62223-SH and DOHH shRNA (m) Lentiviral Particles: sc-62223-V as alternate gene silencing products.

For independent verification of DOHH (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-62223A, sc-62223B and sc-62223C.

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

DOHH siRNA (m) is recommended for the inhibition of DOHH 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

DOHH (E-2): sc-271868 is recommended as a control antibody for monitoring of DOHH 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 DOHH gene expression knockdown using RT-PCR Primer: DOHH (m)-PR: sc-62223-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.