

LLH3 siRNA (h): sc-60952

BACKGROUND

Lysyl hydroxylases (LLHs) 1-3 are hydroxyllysines that function as attachment sites for carbohydrates. In collagen, the LLHs form hydroxyllysine residues in -Xaa-Lys-Gly- sequences and are crucial for collagen crosslink stability. They form homodimers that localize to the endoplasmic reticulum. LLH1 is strongly expressed in liver, heart, lung, skeletal muscle and kidney tissue. LLH2 is highly expressed in heart, lung, kidney, eye, ovary and placenta, whereas LLH3 is expressed mainly in heart, lung, liver and testis. LLH3 is a 738 amino acid, multifunctional enzyme with lysyl hydroxylase, hydroxyllysyl galactosyl-transferase and galactosylhydroxyllysyl glucosyltransferase activities. LLH3 is necessary for the formation of the basement membrane and its gene expression is tightly regulated in adult tissues. Defects in the LLH3 gene commonly lead to heritable disorders of connective tissue and loss of LLH3 results in embryonic lethality.

REFERENCES

1. Heikkinen, J., et al. 2000. Lysyl hydroxylase 3 is a multifunctional protein possessing collagen glucosyltransferase activity. *J. Biol. Chem.* 275: 36158-36163.
2. Wang, C., et al. 2002. Identification of amino acids important for the catalytic activity of the collagen glucosyltransferase associated with the multifunctional lysyl hydroxylase 3 (LLH3). *J. Biol. Chem.* 277: 18568-18573.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 603066. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Rautavuoma, K., et al. 2004. Premature aggregation of Type IV Collagen and early lethal 3 null mice. *Proc. Natl. Acad. Sci. USA* 101: 14120-14125.
5. Risteli, M., et al. 2004. Characterization of collagenous peptides bound to lysyl hydroxylase isoforms. *J. Biol. Chem.* 279: 37535-37543.

CHROMOSOMAL LOCATION

Genetic locus: DDTL (human) mapping to 22q11.23.

PRODUCT

LLH3 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 LLH3 shRNA Plasmid (h): sc-60952-SH and LLH3 shRNA (h) Lentiviral Particles: sc-60952-V as alternate gene silencing products.

For independent verification of LLH3 (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-60952A, sc-60952B and sc-60952C.

PROTOCOLS

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

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

LLH3 siRNA (h) is recommended for the inhibition of LLH3 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

LLH3 (A-10): sc-166007 is recommended as a control antibody for monitoring of LLH3 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 LLH3 gene expression knockdown using RT-PCR Primer: LLH3 (h)-PR: sc-60952-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.