

LLH1 siRNA (m): sc-60949

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 cross-link 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. LLH1 preferentially hydroxylates triple helical lysine residues at the cross-link positions. Decreased levels of LLH1 expression may lead to Ehlers-Danlos syndrome type VI in skin fibroblasts. This syndrome refers to a heterogeneous group of inherited connective tissue disorders that are characterized by joint hypermobility, skin fragility and hyperextensibility.

REFERENCES

1. Passoja, K., et al. 1998. Identification of arginine-700 as the residue that binds the C-5 carboxyl group of 2-oxoglutarate in human lysyl hydroxylase 1. *FEBS Lett.* 434: 145-148.
2. Ruotsalainen, H., et al. 1999. Characterization of cDNAs for mouse lysyl hydroxylase 1, 2 and 3, their phylogenetic analysis and tissue-specific expression in the mouse. *Matrix Biol.* 18: 325-329.
3. Yeowell, H.N., et al. 2000. Deletion of cysteine 369 in lysyl hydroxylase 1 eliminates enzyme activity and causes Ehlers-Danlos syndrome type VI. *Matrix Biol.* 19: 37-46.
4. Yeowell, H.N., et al. 2000. Mutations in the lysyl hydroxylase 1 gene that result in enzyme deficiency and the clinical phenotype of Ehlers-Danlos syndrome type VI. *Mol. Genet. Metab.* 71: 212-224.

CHROMOSOMAL LOCATION

Genetic locus: Plod1 (mouse) mapping to 4 E2.

PRODUCT

LLH1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see LLH1 shRNA Plasmid (m): sc-60949-SH and LLH1 shRNA (m) Lentiviral Particles: sc-60949-V as alternate gene silencing products.

For independent verification of LLH1 (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-60949A, sc-60949B and sc-60949C.

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.

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

LLH1 siRNA (m) is recommended for the inhibition of LLH1 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 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

LLH1 (B-5): sc-271640 is recommended as a control antibody for monitoring of LLH1 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 LLH1 gene expression knockdown using RT-PCR Primer: LLH1 (m)-PR: sc-60949-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.