SANTA CRUZ BIOTECHNOLOGY, INC.

Lubricin siRNA (h): sc-60972

BACKGROUND

Lubricin, also designated proteoglycan-4 or megakaryocyte stimulating factor, plays an important role in boundary lubrication within articulating joints. The disulfide-linked dimer of Lubricin, bonded between Cys 1146 and Cys 1403, is essential for protein cleavage. Highly expressed in cartilage, liver and synovial tissue, Lubricin inhibits synovial cell adhesion to the cartilage surface, but also prevents the deposition of proteins from synovial fluid into cartilage. Defects in the gene encoding for Lubricin can cause Jakobs syndrome, also designated camptodactyly-arthropathy-coxa vara-pericarditis syndrome (CACP). CACP is an autosomal recessive disorder characterized by joint failure associated with noninflammatory synoviocyte hyperplasia and subinitimal fibrosis of the synovial capsule. Lubricin undergoes different levels of glycosylation and may be detected at varying molecular weights.

REFERENCES

1. Schaefer, D.B., et al. 2004. Lubricin reduces cartilage—cartilage integration. Biorheology 41: 503-508.
2. Elsaid, K.A., et al. 2005. Association of articular cartilage degradation and loss of boundary-lubricating ability of synovial fluid following injury and inflammatory arthritis. Arthritis Rheum. 52: 1746-1755.
3. Konttinen, Y.T., et al. 2005. The microenvironment around total hip replacement prostheses. Clin. Orthop. Relat. Res. 430: 28-38.
4. Rhee, D.K., et al. 2005. Consequences of disease-causing mutations on Lubricin protein synthesis, secretion and posttranslational processing. J. Biol. Chem. 280: 31325-31332.
5. Rhee, D.K., et al. 2005. The secreted glycoprotein Lubricin protects cartilage surfaces and inhibits synovial cell overgrowth. J. Clin. Invest. 115: 622-631.

CHROMOSOMAL LOCATION

Genetic locus: PRG4 (human) mapping to 1q31.1.

PRODUCT

Lubricin 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Lubricin shRNA Plasmid (h): sc-60972-SH and Lubricin shRNA (h) Lentiviral Particles: sc-60972-V as alternate gene silencing products.

For independent verification of Lubricin (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-60972A, sc-60972B and sc-60972C.

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 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

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

Lubricin (1D5): sc-293466 is recommended as a control antibody for monitoring of Lubricin 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 Lubricin gene expression knockdown using RT-PCR Primer: Lubricin (h)-PR: sc-60972-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.