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Lumican shRNA (m) Lentiviral Particles: sc-44805-V

BACKGROUND

Lumican, a member of the small leucine-rich proteoglycans (SLRPs) family of proteins, is a keratan sulfate proteoglycan present in large quantities in the corneal stroma and in interstitial collagenous matrices of the heart, aorta, skeletal muscle, skin, and intervertebral discs. During collagen fibrillogenesis, lumican regulates the assembly of collagens into higher order fibrils, limiting the growth of fibrils in diameter. Lumican is present in the extracellular matrix of human articular cartilage at all ages, with a far greater abundance in the adult. The gene encoding human lumican maps to chromosome 12q21.3-q22.

REFERENCES

1. Chakravarti, S., et al. 1995. Primary structure of human lumican (keratan sulfate proteoglycan) and localization of the gene (LUM) to chromosome 12q21.3-q22. *Genomics* 3: 481-488.
2. Chakravarti, S., et al. 1995. Localization of mouse lumican (keratan sulfate proteoglycan) to distal chromosome 10. *Mamm. Genome* 6: 367-368.
3. Grover, J., et al. 1995. The human lumican gene. Organization, chromosomal location, and expression in articular cartilage. *J. Biol. Chem.* 37: 21942-21949.
4. Jepsen, K., et al. 2002. A syndrome of joint laxity and impaired tendon integrity in lumican- and fibromodulin-deficient mice. *J. Biol. Chem.* 38: 35532-35540.
5. Chakravarti, S. 2002. Functions of lumican and fibromodulin: lessons from knockout mice. *Glycoconj. J.* 19: 287-293.
6. Kao, W.W., et al. 2002. Roles of lumican and keratocan on corneal transparency. *Glycoconj. J.* 19: 275-285.

CHROMOSOMAL LOCATION

Genetic locus: Lum (mouse) mapping to 10 C3.

PRODUCT

Lumican shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Lumican siRNA (m): sc-44805 and Lumican shRNA Plasmid (m): sc-44805-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

Lumican shRNA (m) Lentiviral Particles is recommended for the inhibition of Lumican expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Lumican (L-20): sc-27718 is recommended as a control antibody for monitoring of Lumican 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker[™] compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker[™] Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Lumican gene expression knockdown using RT-PCR Primer: Lumican (m)-PR: sc-44805-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.