

aggrecan siRNA (h): sc-41897

BACKGROUND

The large chondroitin sulfate proteoglycan, aggrecan, is the predominant proteoglycan present in cartilage. Aggrecan is a member of the chondroitin sulphate proteoglycan family, which also includes versican/PG-M, neurocan and brevican. Aggrecan is a complex multidomain macromolecule that undergoes extensive processing and posttranslational modification. In cartilage, aggrecan forms aggregates with hyaluronan and link protein, embedded in a collagen network. Aggrecan accounts for the compressive stiffness and resilience of the hyaline cartilage. Many forms of inflammatory arthritis are shown to be accompanied with aggrecan degradation and loss from the cartilage.

REFERENCES

1. Buzas, E.I., et al. 1996. Aggrecan: a target molecule of autoimmune reactions. *Pathol. Oncol. Res.* 2: 219-228.
2. Domowicz, M.S., et al. 2000. Role of the C-terminal G₃ domain in sorting and secretion of aggrecan core protein and ubiquitin-mediated degradation of accumulated mutant precursors. *J. Biol. Chem.* 275: 35098-35105.
3. Knudson, C.B., et al. 2001. Cartilage proteoglycans. *Semin. Cell Dev. Biol.* 12: 69-78.
4. Chen, T.L., et al. 2001. Aggrecan domains expected to traffic through the exocytic pathway are misdirected to the nucleus. *Exp. Cell Res.* 263: 224-235.
5. Kiani, C., et al. 2001. Roles of aggrecan domains in biosynthesis, modification by glycosaminoglycans and product secretion. *Biochem. J.* 354: 199-207.
6. Brückner, G., et al. 2008. Aggrecan-based extracellular matrix is an integral part of the human basal ganglia circuit. *Neuroscience* 151: 489-504.

CHROMOSOMAL LOCATION

Genetic locus: ACAN (human) mapping to 15q26.1.

PRODUCT

aggrecan 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 aggrecan shRNA Plasmid (h): sc-41897-SH and aggrecan shRNA (h) Lentiviral Particles: sc-41897-V as alternate gene silencing products.

For independent verification of aggrecan (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-41897A, sc-41897B and sc-41897C.

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

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

aggrecan (4F4): sc-33695 is recommended as a control antibody for monitoring of aggrecan 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 aggrecan gene expression knockdown using RT-PCR Primer: aggrecan (h)-PR: sc-41897-PR (20 μ l, 586 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.