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SANTA CRUZ BIOTECHNOLOGY, INC.

HAS2 siRNA (h): sc-45328

BACKGROUND

HAS1, HAS2 and HAS3 are HA (hyaluronan or hyaluronic acid) synthase proteins. The extracellular matrix in most vertebrates express HA, which is a high molecular weight linear polysaccharide composed of alternating glucuronic acid and N-acetylglucosamine residues linked by β-1,3 and β-1,4 glycosidic bonds. The three HAS genes show distinct patterns of expression during development and their protein products play significantly different roles in the formation of the HA matrix. Both HAS1 and HAS2 synthesise high molecular-weight HA, whereas HAS3 produces lower molecular weight HA. The expression of the three HAS isoforms is more prominent in growing cells than in resting cells and is differentially regulated by various stimuli suggesting distinct functional roles of the three proteins. HAS2 mRNA shows predominant expression in chondrocytes and cartilage. The human HAS2 gene maps to chromosome 8q24.13.

REFERENCES

1. Spicer, A.P., et al. 1997. Chromosomal localization of the human and mouse hyaluronan synthase genes. Genomics 41: 493-497.
2. Itano, N., et al. 1999. Three isoforms of mammalian hyaluronan synthases have distinct enzymatic properties. J. Biol. Chem. 274: 25085-25092.
3. Jacobson, A., et al. 2000. Expression of human hyaluronan synthases in response to external stimuli. Biochem. J. 348 Pt 1: 29-35.
4. Ijuin, C., et al. 2001. Regulation of hyaluronan synthase gene expression in human periodontal ligament cells by tumour necrosis factor-α, interleukin-1β and interferon-γ. Arch. Oral Biol. 46: 767-772.
5. Recklies, A.D., et al. 2001. Differential regulation and expression of hyaluronan synthases in human articular chondrocytes, synovial cells and osteosarcoma cells. Biochem. J. 354: 17-24.

CHROMOSOMAL LOCATION

Genetic locus: HAS2 (human) mapping to 8q24.13.

PRODUCT

HAS2 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 HAS2 shRNA Plasmid (h): sc-45328-SH and HAS2 shRNA (h) Lentiviral Particles: sc-45328-V as alternate gene silencing products.

For independent verification of HAS2 (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-45328A, sc-45328B and sc-45328C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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

HAS2 siRNA (h) is recommended for the inhibition of HAS2 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

HAS2 (C-5): sc-365263 is recommended as a control antibody for monitoring of HAS2 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 goat anti-mouse IgM-HRP: sc-2064 (dilution range: 1:500-1:5,000), TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgM-FITC: sc-2082 (dilution range: 1:100-1:400) or goat anti-mouse IgM-TR: sc-2983 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor HAS2 gene expression knockdown using RT-PCR Primer: HAS2 (h)-PR: sc-45328-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Guo, N., et al. 2011. Peroxisome proliferator-activated receptor γ ligands inhibit transforming growth factor-β-induced, hyaluronan-dependent, T cell adhesion to orbital fibroblasts. J. Biol. Chem. 286: 18856-18867.