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HAS3 shRNA (m) Lentiviral Particles: sc-45296-V

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 synthesize 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. HAS3 produces both secreted and cell-associated forms of hyaluronan and is the most active of the three isoforms of this enzyme in adults. HAS3 gene expression plays a crucial role in the regulation of hyaluronan synthesis in the epidermis. Specifically, IFN- γ markedly upregulates HAS3 mRNA, whereas TGF β downregulates HAS3 transcript levels. The human HAS3 gene maps to chromosome 16q22.1.

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.* 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-1 β and interferon- γ . *Arch. Oral Biol.* 46: 767-772.

CHROMOSOMAL LOCATION

Genetic locus: Has3 (mouse) mapping to 8 D3.

PRODUCT

HAS3 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 HAS3 siRNA (m): sc-45296 and HAS3 shRNA Plasmid (m): sc-45296-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

HAS3 shRNA (m) Lentiviral Particles is recommended for the inhibition of HAS3 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

HAS3 (L-14): sc-34202 is recommended as a control antibody for monitoring of HAS3 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 HAS3 gene expression knockdown using RT-PCR Primer: HAS3 (m)-PR: sc-45296-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.