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HAS3 siRNA (h): sc-45295

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

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- Jacobson, A., et al. 2000. Expression of human hyaluronan synthases in response to external stimuli. *Biochem. J.* 1: 29-35.
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- Liu, N., et al. 2001. Hyaluronan synthase 3 overexpression promotes the growth of TSU prostate cancer cells. *Cancer Res.* 61: 5207-5214.
- Itano, N., et al. 2002. Abnormal accumulation of hyaluronan matrix diminishes contact inhibition of cell growth and promotes cell migration. *Proc. Natl. Acad. Sci. USA* 99: 3609-3614.
- Sayo, T., et al. 2002. Hyaluronan synthase 3 regulates hyaluronan synthesis in cultured human keratinocytes. *J. Invest. Dermatol.* 118: 43-48.

CHROMOSOMAL LOCATION

Genetic locus: HAS3 (human) mapping to 16q22.1.

PRODUCT

HAS3 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 HAS3 shRNA Plasmid (h): sc-45295-SH and HAS3 shRNA (h) Lentiviral Particles: sc-45295-V as alternate gene silencing products.

For independent verification of HAS3 (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-45295A, sc-45295B and sc-45295C.

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

HAS3 siRNA (h) is recommended for the inhibition of HAS3 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

HAS3 (G-12): sc-365322 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 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 HAS3 gene expression knockdown using RT-PCR Primer: HAS3 (h)-PR: sc-45295-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.