SANTA CRUZ BIOTECHNOLOGY, INC.

Smad5 siRNA (h): sc-38378

BACKGROUND

Smad proteins, the mammalian homologs of the *Drosophila* Mothers against dpp (Mad) have been implicated as downstream effectors of TGFβ/BMP signaling. Smad1 (also designated Madr1 or JV4-1), Smad5 and mammalian Smad8 (also designated Smad9 or MadH6) are effectors of BMP2 and BMP4 function while Smad2 (also designated Madr2 or JV18-1) and Smad3 are involved in TGFβ and Activin-mediated growth modulation. Smad4 (also designated DPC4) has been shown to mediate all of the above activities through interaction with various Smad family members. Smad6 and Smad7 regulate the response to Activin/TGFβ signaling by interfering with TGFβ-mediated phosphorylation of other Smad family members.

CHROMOSOMAL LOCATION

Genetic locus: SMAD5 (human) mapping to 5q31.1.

PRODUCT

Smad5 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 Smad5 shRNA Plasmid (h): sc-38378-SH and Smad5 shRNA (h) Lentiviral Particles: sc-38378-V as alternate gene silencing products.

For independent verification of Smad5 (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-38378A, sc-38378B and sc-38378C.

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

Smad5 siRNA (h) is recommended for the inhibition of Smad5 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

Smad5 (YY-6): sc-101151 is recommended as a control antibody for monitoring of Smad5 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Smad5 gene expression knockdown using RT-PCR Primer: Smad5 (h)-PR: sc-38378-PR (20 µl, 573 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Gupta, A., et al. 2012. Integrin $\alpha_v\beta_3$ and CD44 pathways in metastatic prostate cancer cells support osteoclastogenesis via a Runx2/Smad5/receptor activator of NFκB ligand signaling axis. Mol. Cancer 11: 66.
2. Dai, J., et al. 2013. Genistein promotion of osteogenic differentiation through BMP2/SMAD5/RUNX2 signaling. Int. J. Biol. Sci. 9: 1089-1098.
3. Wang, J., et al. 2014. A heterocyclic molecule kartogenin induces collagen synthesis of human dermal fibroblasts by activating the Smad4/Smad5 pathway. Biochem. Biophys. Res. Commun. 450: 568-574.
4. Nie, M., et al. 2015. MiR-23a and miR-27a promote human granulosa cell apoptosis by targeting SMAD5. Biol. Reprod. 93: 98.
5. Miyakawa, A.A., et al. 2018. Rapamycin activates TGF receptor independently of its ligand: implications for endothelial dysfunction. Clin. Sci. 132: 437-447.
6. Mahdloo, T., et al. 2021. Up-regulation of miR-155 potentiates CD34+ CML stem/progenitor cells to escape from the growth-inhibitory effects of TGF-β1 and BMP signaling. EXCLI J. 20: 748-763.

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.