

Smad5 siRNA (r): sc-63290

BACKGROUND

Smad proteins, the mammalian homologs of the *Drosophila* mothers against dpp (Mad) have been implicated as downstream effectors of TGF β /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 TGF β 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/TGF β signaling by interfering with TGF β -mediated phosphorylation of other Smad family members.

REFERENCES

1. Liu, F., et al. 1996. A human Mad protein acting as a BMP-regulated transcriptional activator. *Nature* 381: 620-623.3.
2. Zhang, Y., et al. 1996. Receptor-associated Mad homologs synergize as effectors of the TGF β response. *Nature* 383: 168-172.
3. Lagna, G., et al. 1996. Partnership between DPC4 and Smad proteins in TGF β signalling pathways. *Nature* 383: 832-836.
4. Eppert, K., et al. 1996. Madr2 maps to 18q21 and encodes a TGF β -regulated Mad-related protein that is functionally encoded in colorectal carcinoma. *Cell* 86: 543-552.
5. Imamura, T., et al. 1997. Smad6 inhibits signalling by the TGF β superfamily. *Nature* 389: 622-626.
6. Heldin, C.H., et al. 1997. TGF β signalling from cell membrane to nucleus through Smad proteins. *Nature* 390: 465-471.
7. Chen, Y., et al. 1997. Smad8 mediates the signaling of the receptor serine kinase. *Proc. Natl. Acad. Sci. USA* 94: 12938-12943.
8. Massagué, J., et al. 1997. TGF β signalling through the Smad pathway. *Trends Cell Biol.* 7: 187-192.

CHROMOSOMAL LOCATION

Genetic locus: Smad5 (rat) mapping to 17q14.

PRODUCT

Smad5 siRNA (r) 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 Smad5 shRNA Plasmid (r): sc-63290-SH and Smad5 shRNA (r) Lentiviral Particles: sc-63290-V as alternate gene silencing products.

For independent verification of Smad5 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-63290A, sc-63290B and sc-63290C.

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

Smad5 siRNA (r) is recommended for the inhibition of Smad5 expression in rat 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

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-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 Smad5 gene expression knockdown using RT-PCR Primer: Smad5 (r)-PR: sc-63290-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.