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Smad4 shRNA (m2) Lentiviral Particles: sc-44274-V

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

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- Zhang, Y., et al. 1996. Receptor-associated Mad homologues synergize as effectors of the TGF β response. *Nature* 383: 168-172.
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- Massaous, J., et al. 1997. TGF β signalling through the Smad pathway. *Trends Cell Biol.* 7: 187-192.
- Chen, Y., et al. 1997. Smad8 mediates the signaling of the ALK-2 receptor serine kinase. *Proc. Natl. Acad. Sci. USA* 94: 12938-12943.
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CHROMOSOMAL LOCATION

Genetic locus: Smad4 (mouse) mapping to 18 E2.

PRODUCT

Smad4 shRNA (m2) 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 Smad4 siRNA (m2): sc-44274 and Smad4 shRNA Plasmid (m2): sc-44274-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.

APPLICATIONS

Smad4 shRNA (m2) Lentiviral Particles is recommended for the inhibition of Smad4 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

Smad4 (B-8): sc-7966 is recommended as a control antibody for monitoring of Smad4 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 IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Smad4 gene expression knockdown using RT-PCR Primer: Smad4 (m2)-PR: sc-44274-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.

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