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Nodal shRNA (h) Lentiviral Particles: sc-45478-V

BACKGROUND

The transforming growth factor β (TGF β) superfamily is composed of numerous growth and differentiation factors, including TGF β 1-3, Mullerian inhibiting substance (MIS), growth/differentiation factor (GDF) 1-9, bone morphogenic protein (BMP) 2-8, glial cell line-derived neurotrophic factor (GDNF), Inhibin α , β -A, β -B and β -C, Lefty and Nodal. Members of the TGF β superfamily are involved in embryonic development and adult tissue homeostasis. Ectodermal cells through the primitive streak delaminate and differentiate into mesoderm during gastrulation. Nodal expression is detectable in the primitive streak at the time of mesoderm formation, indicating a potential role for Nodal in mesoderm formation. Nodal has also been shown to be involved in the direction of heart looping and embryonic turning.

REFERENCES

1. Bellairs, R. 1986. The primitive streak. *Anat. Embryol.* 174: 1-14.
2. Massague, J., et al. 1987. Multiple type- β transforming growth factors and their receptors. *J. Cell Physiol. Suppl.* 5: 43-47.
3. Massague, J. 1990. The transforming growth factor- β family. *Annu. Rev. Cell Biol.* 6: 597-641.
4. Zhou, X., et al. 1993. Nodal is a novel TGF β -like gene expressed in the mouse node during gastrulation. *Nature* 361: 543-547.
5. Collignon, J., et al. 1996. Relationship between asymmetric Nodal expression and the direction of embryonic turning. *Nature* 381: 155-158.
6. McPherron, A.C., et al. 1997. Regulation of skeletal muscle mass in mice by a new TGF β superfamily member. *Nature* 387: 83-90.

CHROMOSOMAL LOCATION

Genetic locus: NODAL (human) mapping to 10q22.1.

PRODUCT

Nodal shRNA (h) 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 Nodal siRNA (h): sc-45478 and Nodal shRNA Plasmid (h): sc-45478-SH as alternate gene silencing products.

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.

APPLICATIONS

Nodal shRNA (h) Lentiviral Particles is recommended for the inhibition of Nodal expression in human 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

Nodal (A-9): sc-377508 is recommended as a control antibody for monitoring of Nodal 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 Nodal gene expression knockdown using RT-PCR Primer: Nodal (h)-PR: sc-45478-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.