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Nodal siRNA (h): sc-45478

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

For independent verification of Nodal (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-45478A, sc-45478B and sc-45478C.

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

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

Nodal (G-10): sc-373910 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 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 Nodal gene expression knockdown using RT-PCR Primer: Nodal (h)-PR: sc-45478-PR (20 μ l, 433 bp). 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.