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Lefty siRNA (h): sc-43894

BACKGROUND

The TGF β superfamily is composed of numerous growth and differentiation factors, including transforming growth factor (TGF) 1, 2 and 3; growth/differentiation factor (GDF) 1-9; Mullerian inhibiting substance (MIS); bone morphogenic protein (BMP) 2-8; glial cell line-derived neurotrophic factor (GDNF); inhibins a, b-A, b-B and b-C; Lefty and Nodal. Members of the TGF superfamily are involved in embryonic development and adult tissue homeostasis. Lefty-A and Lefty-B are homologues of murine Lefty-1 and Lefty-2. Lefty-1 is required for left-right axis determination as a regulator of Lefty-2 and Nodal. It is a secreted protein expressed on the left side of developing embryos. The expression of Lefty-1 is mostly in the prospective floor plate (PFP), although weak expression can be seen in the lateral-plate mesoderm (LPM). It is involved in establishing left-right asymmetry of the organ systems of mammals. Lefty-A plays a role in endometrial bleeding. Mutations in this gene have been associated with left-right axis malformations, particularly in the heart and lungs. Some types of infertility have been associated with dysregulated expression of this gene in the endometrium.

REFERENCES

1. Massague, J., et al. 1987. Multiple type- β transforming growth factors and their receptors. *J. Cell. Physiol.* 5: 43-47.
2. Massague, J. 1990. The transforming growth factor β family. *Annu. Rev. Cell Biol.* 6: 597-641.
3. Meno, C., et al. 1996. Left-right asymmetric expression of the TGF family member Lefty in mouse embryos. *Nature* 381: 151-155.
4. Kothapalli, R., et al. 1997. Detection of eba1, a novel human gene of the transforming growth factor β superfamily association of gene expression with endometrial bleeding. *J. Clin. Invest.* 99: 2342-2350
5. McPherron, A.C., et al. 1997. Regulation of skeletal muscle mass in mice by a new TGF β superfamily member. *Nature* 387: 83-90.
6. Meno, C., et al. 1998 Lefty-1 is required for left-right determination as a regulator of Lefty-2 and Nodal. *Cell* 94: 287-297

CHROMOSOMAL LOCATION

Genetic locus: LEFTY1/LEFTY2 (human) mapping to 1q42.12.

PRODUCT

Lefty 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 Lefty shRNA Plasmid (h): sc-43894-SH and Lefty shRNA (h) Lentiviral Particles: sc-43894-V as alternate gene silencing products.

For independent verification of Lefty (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-43894A, sc-43894B and sc-43894C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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

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

Lefty (D-6): sc-365845 is recommended as a control antibody for monitoring of Lefty 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 Lefty gene expression knockdown using RT-PCR Primer: Lefty (h)-PR: sc-43894-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.