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Nanog siRNA (m): sc-44833

BACKGROUND

Nanog (from "Tir Na Nog", the mythologic Celtic land of the ever young) is a divergent homeodomain protein that directs pluripotency and differentiation of undifferentiated embryonic stem cells. Nanog mRNA is present in pluripotent mouse and human cell lines and absent from differentiated cells. Human Nanog protein shares 52% overall amino acid identity with the mouse protein and 85% identity in the homeodomain. Human Nanog maps to gene locus 12p13.31, whereas mouse Nanog maps to gene loci 6 F2. Murine embryonic Nanog expression is detected in the inner cell mass of the blastocyst. High levels of human Nanog expression have been detected by Northern analysis in the undifferentiated N-Tera embryonal carcinoma cell line.

REFERENCES

- Chambers, I., et al. 2003. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell* 113: 643-655.
- Pan, G.J., et al. 2003. Identification of two distinct transactivation domains in the pluripotency sustaining factor nanog. *Cell Res.* 13: 499-502.
- Online Mendelian Inheritance in Man, OMIM™. 2003. Johns Hopkins University, Baltimore, MD. MIM Number: 607937. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>.
- Hart, A.H., et al. 2004. Identification, cloning and expression analysis of the pluripotency promoting Nanog genes in mouse and human. *Dev. Dyn.* 230: 187-198.
- Clark, A.T., et al. 2004. Human STELLAR, NANOG and GDF3 genes are expressed in pluripotent cells and map to chromosome 12p13, a hotspot for teratocarcinoma. *Stem Cells* 22: 169-179.

CHROMOSOMAL LOCATION

Genetic locus: Nanog (mouse) mapping to 6 F2.

PRODUCT

Nanog siRNA (m) is a pool of 2 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 Nanog shRNA Plasmid (m): sc-44833-SH and Nanog shRNA (m) Lentiviral Particles: sc-44833-V as alternate gene silencing products.

For independent verification of Nanog (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44833A and sc-44833B.

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

Nanog siRNA (m) is recommended for the inhibition of Nanog expression in mouse 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

Nanog (C-4): sc-376915 is recommended as a control antibody for monitoring of Nanog 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 Nanog gene expression knockdown using RT-PCR Primer: Nanog (m)-PR: sc-44833-PR (20 μ l, 591 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Yin, S., et al. 2014. SHP-1 arrests mouse early embryo development through downregulation of Nanog by dephosphorylation of STAT3. *PLoS ONE* 9: e86330.

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.