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Nanog shRNA (h) Lentiviral Particles: sc-43958-V

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 (human) mapping to 12p13.31.

PRODUCT

Nanog shRNA (h) Lentiviral Particles are concentrated, transduction-ready viral particles containing a target-specific construct that encodes a 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl 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 Nanog siRNA (h): sc-43958 and Nanog shRNA Plasmid (h): sc-43958-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

Nanog shRNA (h) Lentiviral Particles is recommended for the inhibition of Nanog expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl 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

Nanog (H-2): sc-374103 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 (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 Nanog gene expression knockdown using RT-PCR Primer: Nanog (h)-PR: sc-43958-PR (20 µl, 536 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Kohler, E.E., et al. 2011. NANOG induction of fetal liver kinase-1 (FLK1) transcription regulates endothelial cell proliferation and angiogenesis. *Blood* 117: 1761-1769.

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.