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Oct-3/4 siRNA (h2): sc-43980

BACKGROUND

POU5F1 (POU domain, class 5, transcription factor 1), also known as octamer-binding transcription factor-3 (Oct-3, Otf-3), octamer-binding transcription factor-4 (Oct-4, Otf-4) and Oct-3/4, modulates embryonic stem (ES) cell populations by influencing lineage commitment. Oct-3/4 sustains stem-cell self-renewal and differentiation pathways. Transcription factors containing the POU homeodomain regulate tissue-specific gene expression in lymphoid and pituitary differentiation and in early mammalian development. Oct-3/4 is capable of inducing rapid proliferation and tumorigenic properties of ES cells through activation of the UTF1 gene. In humans, two Oct-3/4 isoforms contribute to influencing the undifferentiated phenotype of ES cells. Oct-3/4 pseudogenes localizing to human chromosomes 10 and 8 are reported to be transcribed in certain cancer cell lines and tissues.

REFERENCES

1. Takeda, J., et al. 1992. Human Oct-3 gene family: cDNA sequences, alternative splicing, gene organization, chromosomal location, and expression at low levels in adult tissues. *Nucleic Acids Res.* 20: 4613-4620.
2. Nichols, J., et al. 1998. Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct-4. *Cell* 95: 379-391.
3. Niwa, H., et al. 2000. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat. Genet.* 24: 372-376.
4. Nishimoto, M., et al. 2005. Oct-3/4 maintains the proliferative embryonic stem cell state via specific binding to a variant octamer sequence in the regulatory region of the UTF1 locus. *Mol. Cell. Biol.* 25: 5084-5094.

CHROMOSOMAL LOCATION

Genetic locus: POU5F1 (human) mapping to 6p21.33.

PRODUCT

Oct-3/4 siRNA (h2) 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 Oct-3/4 shRNA Plasmid (h2): sc-43980-SH and Oct-3/4 shRNA (h2) Lentiviral Particles: sc-43980-V as alternate gene silencing products.

For independent verification of Oct-3/4 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-43980A, sc-43980B and sc-43980C.

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

Oct-3/4 siRNA (h2) is recommended for the inhibition of Oct-3/4 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

Oct-3/4 (C-10): sc-5279 is recommended as a control antibody for monitoring of Oct-3/4 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 Oct-3/4 gene expression knockdown using RT-PCR Primer: Oct-3/4 (h2)-PR: sc-43980-PR (20 μ l, 512 bp). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

SELECT PRODUCT CITATIONS

1. Wang, X.Q., et al. 2010. Octamer 4 (Oct-4) mediates chemotherapeutic drug resistance in liver cancer cells through a potential Oct4-AKT-ATP-binding cassette G₂ pathway. *Hepatology* 52: 528-539.

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.