

LEF-1 siRNA (m): sc-35805

BACKGROUND

The TCF (T cell factor) family of transcription factors are activated by the Wnt-1 and Wntless pathways and are characterized by the presence of a conserved protein motif, the high mobility group (HMG) 1 box, which mediates DNA binding. The TCF (T cell factor) proteins, which are required during development, include TCF-1, which is essential for lymphoid cell development, and TCF-3 and TCF-4, which are implicated in neuronal development and LEF (leukemia enhancer factor). The Wnt mediated signaling pathway induces cytosolic β -catenin binding to TCF proteins within the nucleus, leading to the enhanced expression of the Wnt target genes. The β -catenin-TCF complexes are negatively regulated by the adenomatous polyposis coli (APC) tumor suppressor protein, which phosphorylates β -catenin and, in turn, increases the degradation of cytosolic β -catenin to, thereby, inhibit the transcriptional activity of the TCF proteins. Mutations in the APC gene, which are commonly observed in colorectal carcinomas, disrupt this regulatory pathway and correlate to an accumulation of β -catenin and the increased activation of the TCF target genes.

REFERENCES

1. van de Wetering, M., et al. 1991. Identification and cloning of TCF-1, a T lymphocyte-specific transcription factor containing a sequence-specific HMG box. *EMBO J.* 10: 123-132.
2. van de Wetering, M., et al. 1992. The human T cell transcription factor-1 gene. Structure, localization, and promoter characterization. *J. Biol. Chem.* 267: 8530-8536.
3. Verbeek, S., et al. 1995. An HMG-box-containing T cell factor required for thymocyte differentiation. *Nature* 374: 70-74.

CHROMOSOMAL LOCATION

Genetic locus: Lef1 (mouse) mapping to 3 G3.

PRODUCT

LEF-1 siRNA (m) 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 LEF-1 shRNA Plasmid (m): sc-35805-SH and LEF-1 shRNA (m) Lentiviral Particles: sc-35805-V as alternate gene silencing products.

For independent verification of LEF-1 (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-35805A, sc-35805B and sc-35805C.

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

LEF-1 siRNA (m) is recommended for the inhibition of LEF-1 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

LEF-1 (B-6): sc-374522 is recommended as a control antibody for monitoring of LEF-1 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 LEF-1 gene expression knockdown using RT-PCR Primer: LEF-1 (m)-PR: sc-35805-PR (20 μ l, 460 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Yun, K. and Im, S.H. 2007. Lef1 regulates Cox-2 transcription in chondrocytes. *Biochem. Biophys. Res. Commun.* 364: 270-275.
2. Yun, K. and Im, S.H. 2007. Transcriptional regulation of MMP13 by LEF-1 in chondrocytes. *Biochem. Biophys. Res. Commun.* 364: 1009-1014.
3. Jash, A., et al. 2012. Looping mediated interaction between the promoter and 3' UTR regulates type II collagen expression in chondrocytes. *PLoS ONE* 7: e40828.
4. Cironi, L., et al. 2016. The fusion protein SS18-SSX1 employs core Wnt pathway transcription factors to induce a partial Wnt signature in synovial sarcoma. *Sci. Rep.* 6: 22113.

RESEARCH USE

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