

TLE4 siRNA (h): sc-38562

BACKGROUND

The Notch signaling pathway controls cellular interactions important for the specification of a variety of fates in both invertebrates and vertebrates. Key players in the Notch pathway are the TLE genes (for transducin-like enhancer of split, also designated ESG for enhancer of split Groucho), which are human homologs of the *Drosophila* Groucho gene. Groucho is a transcriptional repressor that plays a key role in neurogenesis, segmentation and sex determination. TLEs associate with chromatin in live cells and specifically with Histone H3, but not with other core histones. Expression of the TLE genes, TLE1, TLE2, TLE3 and TLE4, correlate with immature epithelial cells that are progressing toward a terminally differentiated state, suggesting a role during epithelial differentiation. TLE1, TLE2 and TLE3 have elevated expression in cervical squamous metaplasias and carcinomas, while TLE4 is most highly expressed in the brain, particularly in the caudate nucleus. TLE1 and TLE4 contain SP and WD40 domains, through which TLE1 binds AML1 to inhibit AML1-induced transactivation of the CSF1 receptor. In early stages of cell differentiation, TLE1 is upregulated, and TLE2 and TLE4 are downregulated. In later stages, TLE2 and TLE4 are upregulated, and expression of TLE1 decreases.

REFERENCES

1. Stifani, S., et al. 1992. Human homologs of a *Drosophila* Enhancer of split gene product define a novel family of nuclear proteins. *Nat. Genet.* 2: 119-127.
2. Paroush, Z., et al. 1994. Groucho is required for *Drosophila* neurogenesis, segmentation, and sex determination and interacts directly with hairy-related bHLH proteins. *Cell* 79: 805-815.
3. Liu, Y., et al. 1996. Epithelial expression and chromosomal location of human TLE genes: implications for notch signaling and neoplasia. *Genomics* 31: 58-64.
4. Palaparti, A., et al. 1997. The Groucho/transducin-like enhancer of split transcriptional repressors interact with the genetically defined amino-terminal silencing domain of Histone H3. *J. Biol. Chem.* 272: 26604-26610.
5. Levanon, D., et al. 1998. Transcriptional repression by AML1 and LEF-1 is mediated by the TLE/Groucho corepressors. *Proc. Natl. Acad. Sci. USA* 95: 11590-11595.

CHROMOSOMAL LOCATION

Genetic locus: TLE4 (human) mapping to 9q21.31.

PRODUCT

TLE4 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 TLE4 shRNA Plasmid (h): sc-38562-SH and TLE4 shRNA (h) Lentiviral Particles: sc-38562-V as alternate gene silencing products.

For independent verification of TLE4 (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-38562A, sc-38562B and sc-38562C.

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

TLE4 siRNA (h) is recommended for the inhibition of TLE4 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

TLE4 (E-10): sc-365406 is recommended as a control antibody for monitoring of TLE4 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 TLE4 gene expression knockdown using RT-PCR Primer: TLE4 (h)-PR: sc-38562-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.