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SANTA CRUZ BIOTECHNOLOGY, INC.

TLF siRNA (h): sc-44158

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

The TATA box-binding protein (TBP) is an essential component of the basal transcriptional machinery. TBP and the various RNA polymerase subunits are assembled with unique TBP-associated factors (TAFs) into distinct complexes that act specifically with either RNA polymerase I (SL1/TIF-IB), RNA polymerase II (TFIID), or RNA polymerase III (TFIIIB) on cognate promoters. TLF, also called TBP-related factor 2 (TRF2), activates a number of different genes, including the neurofibromatosis type 1 (NF1) gene. TLF is related in sequence and structure to TBP and the *Drosophila* TBP-related factor TRF1. TLF functions as gene-specific factor for RNA polymerase II-mediated transcription, but unlike TBP, TLF does not appear to be universal binding factor of other RNA polymerase complexes. TLF preferentially binds to and forms a stable complex with TFIIA. TFIIA is required as a core promoter selective factor for both basal and activated TFIID-mediated transcription as it enhances TBP/TFIID binding to DNA and alleviates TFIID repression that is mediated by negative cofactors.

REFERENCES

1. Meisterernst, M., et al. 1991. Family of proteins that interact with TFIID and regulate promoter activity. Cell 67: 557-567.
2. Orphanides, G., et al. 1996. The general transcription factors of RNA polymerase II. Genes Dev. 10: 2657-2683.
3. Lee, T.I., et al. 1998. Regulation of gene expression by TBP-associated proteins. Genes Dev. 12: 1398-1408.
4. Ozer, J., et al. 1998. Transcription factor IIA derepresses TATA-binding protein (TBP)-associated factor inhibition of TBP-DNA binding. J. Biol. Chem. 273: 14293-14300.
5. Rabenstein, M.D., et al. 1999. TATA box-binding protein (TBP)-related factor 2 (TRF2), a third member of the TBP family. Proc. Natl. Acad. Sci. USA 96: 4791-4796.
6. Teichmann, M., et al. 1999. Human TATA-binding protein-related factor 2 (hTRF2) stably associates with hTFIIA in HeLa cells. Proc. Natl. Acad. Sci. USA 96: 13720-13725.
7. Maldonado, E. 1999. Transcriptional functions of a new mammalian TATA-binding protein-related factor. J. Biol. Chem. 274: 12963-12966.

CHROMOSOMAL LOCATION

Genetic locus: TBPL1 (human) mapping to 6q23.2.

PRODUCT

TLF 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TLF shRNA Plasmid (h): sc-44158-SH and TLF shRNA (h) Lentiviral Particles: sc-44158-V as alternate gene silencing products.

For independent verification of TLF (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-44158A, sc-44158B and sc-44158C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

TLF siRNA (h) is recommended for the inhibition of TLF 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 µM in 66 µl. 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

TLF (C-8): sc-514059 is recommended as a control antibody for monitoring of TLF 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 TLF gene expression knockdown using RT-PCR Primer: TLF (h)-PR: sc-44158-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72°

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.