

TRFP siRNA (h): sc-38598

BACKGROUND

In mammalian cells, transcription is regulated in part by high molecular weight coactivating complexes that mediate signals between transcriptional activators and RNA polymerase. These complexes include SMCC (Srb and MED protein cofactor complex), which consists of various subunits that share homology with several components of the yeast transcriptional mediator complexes. SMCC associates with the RNAPII (RNA polymerase II) holoenzyme through Srb7 and, in turn, enhances gene-specific activation or repression induced by DNA-binding transcription factors. Srb7 also interacts with an additional member of the RNAPII holoenzyme, the human homolog of *Drosophila* TBP-related factor (TRF)-proximal protein (TRFP). TRFP synergistically associates with coactivators, including PC4 (positive coactivator 4) and USA (upstream stimulatory activity) of the RNAPII and SMCC complex, to enhance basal and gene-specific transcription.

REFERENCES

1. Aragona, M., et al. 2000. Immunohistochemical telomeric-repeat binding factor-1 expression in gastrointestinal tumors. *Oncol. Rep.* 7: 987-990.
2. Matsutani, N., et al. 2001. Expression of telomeric repeat binding factor 1 and 2 and TRF1-interacting nuclear protein 2 in human gastric carcinomas. *Int. J. Oncol.* 19: 507-512.
3. Yajima, T., et al. 2001. Telomerase reverse transcriptase and telomeric-repeat binding factor protein 1 as regulators of telomerase activity in pancreatic cancer cells. *Br. J. Cancer* 85: 752-757.
4. Seimiya, H., et al. 2002. The telomeric poly(ADP-ribose) polymerase, tankyrase 1, contains multiple binding sites for telomeric repeat binding factor 1 (TRF1) and a novel acceptor, 182-kDa tankyrase-binding protein (TAB182). *J. Biol. Chem.* 277: 14116-14126.
5. Nakanishi, K., et al. 2003. Expression of mRNAs for telomeric repeat binding factor (TRF)-1 and TRF2 in atypical adenomatous hyperplasia and adenocarcinoma of the lung. *Clin. Cancer Res.* 9: 1105-1111.
6. Yang, S.W., et al. 2003. Expression of the telomeric repeat binding factor gene NgTRF1 is closely coordinated with the cell division program in tobacco BY-2 suspension culture cells. *J. Biol. Chem.* 278: 21395-21407.

CHROMOSOMAL LOCATION

Genetic locus: MED20 (human) mapping to 6p21.1.

PRODUCT

TRFP 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 TRFP shRNA Plasmid (h): sc-38598-SH and TRFP shRNA (h) Lentiviral Particles: sc-38598-V as alternate gene silencing products.

For independent verification of TRFP (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-38598A, sc-38598B and sc-38598C.

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

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

TRFP (G-11): sc-374247 is recommended as a control antibody for monitoring of TRFP 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 TRFP gene expression knockdown using RT-PCR Primer: TRFP (h)-PR: sc-38598-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.