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TERT siRNA (h2): sc-156050

BACKGROUND

Telomerase is an RNA-dependent DNA polymerase that catalyzes the addition of telomeric repeat sequences to chromosome ends. In most human somatic cells, telomerase activity is undetectable, and telomeres shorten with successive cell divisions. However, telomerase activity is detectable in immortal cells and in many human tumors. Two candidate mammalian telomerase proteins have been cloned. Human TP1 (for telomerase-associated protein 1), also designated TLP1 in rat (for telomerase protein component 1), is homologous to the tetrahymena p80 telomerase protein and has been shown to interact with mammalian telomerase RNA. Human TERT (for telomerase reverse transcriptase), also designated hEST2 (for ever shorter telomeres), is homologous to the p123 telomerase protein from *Euplotes* and to the yeast Est2 protein. Expression of TERT mRNA has been shown to correlate with telomerase activity in various cell lines.

REFERENCES

- Counter, C.M., et al. 1992. Telomere shortening associated with chromosome instability is arrested in immortal cells which express telomerase activity. *EMBO J.* 11: 1921-1929.
- Kim, N.W., et al. 1994. Specific association of human telomerase activity with immortal cells and cancer. *Science* 266: 2011-2015.
- Greider, C.W. 1996. Telomere length regulation. *Annu. Rev. Biochem.* 65: 337-365.
- Harrington, L., et al. 1997. A mammalian telomerase-associated protein. *Science* 275: 973-977.
- Nakamura, T.M., et al. 1997. Telomerase catalytic subunit homologs from fission yeast and human. *Science* 277: 955-959.
- Nakayama, J., et al. 1997. TLP1: a gene encoding a protein component of mammalian telomerase is a novel member of WD repeats family. *Cell* 88: 875-884.
- Meyerson, M., et al. 1997. hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. *Cell* 90: 785-795.

CHROMOSOMAL LOCATION

Genetic locus: TERT (human) mapping to 5p15.33.

PRODUCT

TERT siRNA (h2) is a pool of 2 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 TERT shRNA Plasmid (h2): sc-156050-SH and TERT shRNA (h2) Lentiviral Particles: sc-156050-V as alternate gene silencing products.

For independent verification of TERT (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-156050A and sc-156050B.

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

TERT siRNA (h2) is recommended for the inhibition of TERT 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

TERT (A-6): sc-393013 is recommended as a control antibody for monitoring of TERT 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 TERT gene expression knockdown using RT-PCR Primer: TERT (h2)-PR: sc-156050-PR (20 μ l, 504 bp). 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.