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SANTA CRUZ BIOTECHNOLOGY, INC.

TS shRNA (h) Lentiviral Particles: sc-44978-V

BACKGROUND

Thymidylate synthase (TS), also designated TYMS, TMS, TSase and HsT422, uses 5,10-methylenetetrahydrofolate (methylene-THF) as a cofactor in the synthesis of 2'-deoxythymidine-5'-monophosphate (dTMP), an essential precursor for DNA biosynthesis. TS is an RNA-binding protein that can interact with its own mRNA. The TS/mRNA ribonucleoprotein complex can also associate with a number of other cellular mRNAs, including those corresponding to the p53 tumor suppressor gene and the Myc family of transcription factors. Inhibition of DNA replication and cell death resulting from thymidine depletion occurs when TS enzyme activity is inhibited with substrate or cofactor analogs, making the TS enzyme an important target for chemotherapy. Cancer cells are sensitive to thymidine depletion, as they multiply rapidly.

REFERENCES

1. Hardy, L.W., et al. 1987. Atomic structure of thymidylate synthase: target for rational drug design. Science 235: 448-455.
2. Ross, P., et al. 1990. Cloning and characterization of the thymidylate synthase gene from *Lactococcus lactis* subsp. *lactis*. Appl. Environ. Microbiol. 56: 2156-2163.
3. Kaneda, S., et al. 1990. Structural and functional analysis of the human thymidylate synthase gene. J. Biol. Chem. 265: 20277-20284.
4. Horikoshi, T., et al. 1992. Quantitation of thymidylate synthase, dihydrofolate reductase and DT-diaphorase gene expression in human tumors using the polymerase chain reaction. Cancer Res. 52: 108-116.
5. Johnston, P.G., et al. 1995. Thymidylate synthase gene and protein expression correlate and are associated with response to 5-fluorouracil in human colorectal and gastric tumors. Cancer Res. 55: 1407-1412.

CHROMOSOMAL LOCATION

Genetic locus: TYMS (human) mapping to 18p11.32.

PRODUCT

TS shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see TS siRNA (h): sc-44978 and TS shRNA Plasmid (h): sc-44978-SH as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

TS shRNA (h) Lentiviral Particles is recommended for the inhibition of TS expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

TS (F-7): sc-376161 is recommended as a control antibody for monitoring of TS 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TS gene expression knockdown using RT-PCR Primer: TS (h)-PR: sc-44978-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.