

TS siRNA (m): sc-44979

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

- Hardy, L.W., et al. 1987. Atomic structure of thymidylate synthase: target for rational drug design. *Science* 235: 448-455.
- Ross, P., et al. 1990. Cloning and characterization of the thymidylate synthase gene from *Lactococcus lactis* subsp. *lactis*. *Appl. Environ. Microbiol.* 56: 2156-2163.
- Kaneda, S., et al. 1990. Structural and functional analysis of the human thymidylate synthase gene. *J. Biol. Chem.* 265: 20277-20284.
- Horikoshi, T., et al. 1992. Quantitation of thymidylate synthase, dihydrofolate reductase and DT-diphosphorase gene expression in human tumors using the polymerase chain reaction. *Cancer Res.* 52: 108-116.
- 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.
- Johnston, P.G., et al. 1995. The role of thymidylate synthase expression in prognosis and outcome of adjuvant chemotherapy in patients with rectal cancer. *J. Clin. Oncol.* 12: 2640-2647.

CHROMOSOMAL LOCATION

Genetic locus: *Tyms* (mouse) mapping to 5 B1.

PRODUCT

TS siRNA (m) 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 TS shRNA Plasmid (m): sc-44979-SH and TS shRNA (m) Lentiviral Particles: sc-44979-V as alternate gene silencing products.

For independent verification of TS (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44979A, sc-44979B and sc-44979C.

PROTOCOLS

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

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

TS siRNA (m) is recommended for the inhibition of TS expression in mouse 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

TS (C-5): sc-390945 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 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 TS gene expression knockdown using RT-PCR Primer: TS (m)-PR: sc-44979-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.