SANTA CRUZ BIOTECHNOLOGY, INC.

ENOSF1 siRNA (h): sc-77275

BACKGROUND

Thymidylate synthase (also designated TS, TYMS, TMS, TSase, 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 as well as with a number of other cellular mRNAs. 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. ENOSF1, also designated Antisense RNA to thymidylate synthase or rTS, is a 443 amino acid mitochondrial protein that functions to synthesize signaling molecules that downregulate thymidylate synthase. Existing as three isoforms (α, β and γ), ENOSF1 may be regulated by phosphorylation and/or sumoylation.

REFERENCES

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2. Johnson, L.F. 1994. Posttranscriptional regulation of thymidylate synthase gene expression. J. Cell. Biochem. 54: 387-392.
3. Dolnick, B.J., Black, A.R., Winkler, P.M., Schindler, K. and Hsueh, C.T. 1996. rTS gene expression is associated with altered cell sensitivity to thymidylate synthase inhibitors. Adv. Enzyme Regul. 36: 165-180.
4. Chu, J. and Dolnick, B.J. 2002. Natural antisense (rTSα) RNA induces site-specific cleavage of thymidylate synthase mRNA. Biochim. Biophys. Acta 1587: 183-193.
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6. Dolnick, B.J., Angelino, N.J., Dolnick, R. and Sufrin, J.R. 2003. A novel function for the rTS gene. Cancer Biol. Ther. 2: 364-369.
7. Liang, P., Nair, J.R., Song, L., McGuire, J.J. and Dolnick, B.J. 2005. Comparative genomic analysis reveals a novel mitochondrial isoform of human rTS protein and unusual phylogenetic distribution of the rTS gene. BMC Genomics 6: 125.
8. Dolnick, R., Wu, Q., Angelino, N.J., Stephanie, L.V., Chow, K.C., Sufrin, J.R. and Dolnick, B.J. 2005. Enhancement of 5-fluorouracil sensitivity by an rTS signaling mimic in H630 colon cancer cells. Cancer Res. 65: 5917-5924.
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CHROMOSOMAL LOCATION

Genetic locus: ENOSF1 (human) mapping to 18p11.32.

PROTOCOLS

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

PRODUCT

ENOSF1 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 ENOSF1 shRNA Plasmid (h): sc-77275-SH and ENOSF1 shRNA (h) Lentiviral Particles: sc-77275-V as alternate gene silencing products.

For independent verification of ENOSF1 (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-77275A, sc-77275B and sc-77275C.

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

ENOSF1 siRNA (h) is recommended for the inhibition of ENOSF1 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ENOSF1 gene expression knockdown using RT-PCR Primer: ENOSF1 (h)-PR: sc-77275-PR (20 µl). 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.