

dUTPase siRNA (h): sc-62242

BACKGROUND

dUTPase (deoxyuridine 5'-triphosphate nucleotidohydrolase), also known as DUT or dUTP pyrophosphatase, is a preventive DNA repair enzyme that functions in nucleotide metabolism. dUTPase is expressed in a variety of tissues and, depending on the isoform (DUT-N or DUT-M), localizes to the nucleus or the mitochondrion. The nuclear isoform, DUT-N, is the most abundant of the two isoforms. dUTPase, in the presence of magnesium ions, is responsible for hydrolyzing dUTP to dUMP and diphosphate. This reaction is important for keeping the intracellular dUTP concentration low so that uracil does not become incorporated into DNA. Extensive incorporation of uracil into DNA can ultimately lead to cell death. This suggests that dUTPase is essential for cell viability, further implying that dUTPase is a potential target for anticancer therapy. In addition, dUMP, the product of the hydrolysis reaction, is a precursor of thymidine nucleotides which are essential for DNA replication.

REFERENCES

1. Canman, C.E., et al. 1992. Variations in patterns of DNA damage induced in human colorectal tumor cells by 5-fluorodeoxyuridine: implications for mechanisms of resistance and cytotoxicity. *Proc. Natl. Acad. Sci. USA* 89: 10474-10478.
2. Ladner, R.D., et al. 1996. Characterization of distinct nuclear and mitochondrial forms of human deoxyuridine triphosphate nucleotidohydrolase. *J. Biol. Chem.* 271: 7745-7751.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 601266. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Jiang, Y.L., et al. 2006. Synthesis and high-throughput evaluation of triske-lion uracil libraries for inhibition of human dUTPase and UNG2. *Bioorg. Med. Chem.* 14: 5666-5672.

CHROMOSOMAL LOCATION

Genetic locus: DUT (human) mapping to 15q21.1.

PRODUCT

dUTPase siRNA (h) is a target-specific 19-25 nt siRNA 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 dUTPase shRNA Plasmid (h): sc-62242-SH and dUTPase shRNA (h) Lentiviral Particles: sc-62242-V as alternate gene silencing 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

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

dUTPase (H-9): sc-166856 is recommended as a control antibody for monitoring of dUTPase 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 dUTPase gene expression knockdown using RT-PCR Primer: dUTPase (h)-PR: sc-62242-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Ji, D., et al. 2018. ATP-linked chimeric nucleotide as a specific luminescence reporter of deoxyuridine triphosphatase. *Bioconjug. Chem.* 29: 1614-1621.

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.