SANTA CRUZ BIOTECHNOLOGY, INC.

MTAP siRNA (h): sc-60006

BACKGROUND

5'-deoxy-5'-methylthioadenosine phosphorylase (MTAP, MSAP) catalyzes the reversible phosphorolysis of methylthioadenosine, which is important in polyamine metabolism and for the salvage of adenine and methionine. The gene encoding MTAP maps to human chromosome 9p21.3 and is linked to the tumor suppressor gene, p16INK4A. Deficient levels of MTAP can occur in cancers primarily through co-deletion of the MTAP gene and the p16INK4A gene. Cells expressing MTAP and possessing adenine salvage pathway activity may be less susceptible to malignancy due to growth-inhibitory actions of agents (e.g. antifolates), whose mechanism of action, in part, involves this *de novo* purine pathway.

REFERENCES

1. Nobori, T., et al. 1996. Genomic cloning of methylthioadenosine phosphorylase: a purine metabolic enzyme deficient in multiple different cancers. Proc. Natl. Acad. Sci. USA 93: 6203-6208.
2. Chen, Z.H., et al. 1997. Expression of methylthioadenosine phosphorylase cDNA in p16-, MTAP-malignant cells: restoration of methylthioadenosine phosphorylase-dependent salvage pathways and alterations of sensitivity to inhibitors of purine *de novo* synthesis. Mol. Pharmacol. 52: 903-911.
3. Yu, J., et al. 1997. Presence of methylthioadenosine phosphorylase (MTAP) in hematopoietic stem/progenitor cells: its therapeutic implication for MTAP-malignancies. Clin. Cancer Res. 3: 433-438.
4. Schmid, M., et al. 1998. Homozygous deletions of methylthioadenosine phosphorylase (MTAP) are more frequent than p16INK4A (CDKN2) homozygous deletions in primary non-small cell lung cancers (NSCLC). Oncogene 17: 2669-2675.
5. Online Mendelian Inheritance in Man, OMIM™. 1999. Johns Hopkins University, Baltimore, MD. MIM Number: 156540. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
6. Schmid, M., et al. 2000. A methylthioadenosine phosphorylase (MTAP) fusion transcript identifies a new gene on chromosome 9p21 that is frequently deleted in cancer. Oncogene 19: 5747-5754.
7. LocusLink Report (LocusID: 4507). http://www.ncbi.nlm.nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: MTAP (human) mapping to 9p21.3.

PRODUCT

MTAP 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 MTAP shRNA Plasmid (h): sc-60006-SH and MTAP shRNA (h) Lentiviral Particles: sc-60006-V as alternate gene silencing products.

For independent verification of MTAP (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-60006A, sc-60006B and sc-60006C.

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

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

GENE EXPRESSION MONITORING

MTAP (42-T): sc-100782 is recommended as a control antibody for monitoring of MTAP 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 MTAP gene expression knockdown using RT-PCR Primer: MTAP (h)-PR: sc-60006-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.

PROTOCOLS

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