SANTA CRUZ BIOTECHNOLOGY, INC.

PAO siRNA (m): sc-44541

BACKGROUND

Mammalian polyamine catabolism is under the control of two enzymes, spermidine/spermine N1-acetyltransferase and the flavin adenine dinucleotide-dependent polyamine oxidase (PAO). In the polyamine back-conversion pathway, spermine and spermidine are acetylated by SSAT-1 and then oxidized by PAO to produce spermidine and putrescine, respectively. The PAO protein regulates polyamine intracellular concentration and may act as a determinant of cellular sensitivity to the antitumor polyamine analogs. PAO contributes to β-alaline production via aldehyde dehydrogenase conversion of 3-aminopropanal. The PAO gene encodes more than five transcript variants which encode four active isoenzymes. The longest isoenzyme, PAOh1, represents a new addition to the polyamine metabolic pathway and may be a target for antineoplastic drug development.

REFERENCES

1. Parry, L., et al. 1995. Effect of expression of human spermidine/spermine N1-acetyltransferase in *Escherichia coli*. Biochemistry 34: 2701-2709.
2. Vujcic, S., et al. 2002. Identification and characterization of a novel flavin-containing spermine oxidase of mammalian cell origin. Biochem. J. 367: 665-675.
3. Wang, Y., et al. 2003. Properties of purified recombinant human polyamine oxidase, PAOh1/SMO. Biochem. Biophys. Res. Commun. 304: 605-611.
4. Chen, Y., et al.. 2003. Genomic identification and biochemical characterization of a second spermidine/spermine N1-acetyltransferase. Biochem. J. 373: 661-667.
5. Pledgie, A., et al. 2005. Spermine oxidase SMO(PAOh1), Not N1-acetylpolyamine oxidase PAO, is the primary source of cytotoxic H_2O_2 in polyamine analogue-treated human breast cancer cell lines. J. Biol. Chem. 280: 39843-39851.
6. Babbar, N. and Casero, R.A., Jr. 2006. Tumor necrosis factor-α increases reactive oxygen species by inducing spermine oxidase in human lung epithelial cells: a potential mechanism for inflammation-induced carcinogenesis. Cancer Res. 66: 11125-11130.

CHROMOSOMAL LOCATION

Genetic locus: Smox (mouse) mapping to 2 F1.

PRODUCT

PAO 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PAO shRNA Plasmid (m): sc-44541-SH and PAO shRNA (m) Lentiviral Particles: sc-44541-V as alternate gene silencing products.

For independent verification of PAO (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-44541A, sc-44541B and sc-44541C.

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

PAO siRNA (m) is recommended for the inhibition of PAO 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 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

PAO (C-3): sc-166185 is recommended as a control antibody for monitoring of PAO 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 PAO gene expression knockdown using RT-PCR Primer: PAO (m)-PR: sc-44541-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.