SANTA CRUZ BIOTECHNOLOGY, INC.

PTEN siRNA (m2): sc-44324

BACKGROUND

As human tumors progress to advanced stages, one genetic alteration that occurs at high frequency is a loss of heterozygosity (LOH) at chromosome 10q23. Mapping of homozygous deletions on this chromosome led to the isolation of the PTEN gene, also designated MMAC1 (for mutated in multiple advanced cancers) and TEP1. This candidate tumor suppressor gene exhibits a high frequency of mutations in human glioblastomas and is also mutated in other cancers, including sporadic brain, breast, kidney and prostate cancers. PTEN has been associated with Cowden disease, an autosomal dominant cancer predisposition syndrome. The PTEN gene product is a putative protein tyrosine phosphatase that is localized to the cytoplasm and shares extensive homology with the cytoskeletal proteins tensin and auxilin. Gene transfer studies have indicated that the phosphatase domain of PTEN is essential for growth suppression of glioma cells.

REFERENCES

1. Bigner, S.H., et al. 1988. Specific chromosomal abnormalities in malignant human gliomas. Cancer Res. 48: 405-411.
2. James, C.D., et al. 1988. Clonal genomic alterations in glioma malignancy stages. Cancer Res. 48: 5546-5551.
3. Steck, P.A., et al. 1997. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat. Genet. 15: 356-362.
4. Li, J., et al. 1997. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 275: 1943-1947.
5. Liaw, D., et al. 1997. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat. Genet. 16: 64-67.
6. Nelen, M.R., et al. 1997. Germline mutations in the PTEN/MMAC1 gene in patients with Cowden disease. Hum. Mol. Genet. 6: 1383-1387.
7. Fumari, F.B., et al. 1997. Growth suppression of glioma cells by PTEN requires a functional phosphatase catalytic domain. Proc. Natl. Acad. Sci. USA 94: 12479-12484.

CHROMOSOMAL LOCATION

Genetic locus: Pten (mouse) mapping to 19 C1.

PRODUCT

PTEN siRNA (m2) 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 PTEN shRNA Plasmid (m2): sc-44324-SH and PTEN shRNA (m2) Lentiviral Particles: sc-44324-V as alternate gene silencing products.

For independent verification of PTEN (m2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44324A, sc-44324B and sc-44324C.

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

PTEN siRNA (m2) is recommended for the inhibition of PTEN 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

PTEN (A2B1): sc-7974 is recommended as a control antibody for monitoring of PTEN 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 PTEN gene expression knockdown using RT-PCR Primer: PTEN (m2)-PR: sc-44324-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.