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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 



PTEN shRNA (h2) Lentiviral Particles: sc-44272-V

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. 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 (human) mapping to 10q23.31.

PRODUCT

PTEN shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see PTEN siRNA (h2): sc-44272 and PTEN shRNA Plasmid (h2): sc-44272-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

PTEN shRNA (h2) Lentiviral Particles is recommended for the inhibition of PTEN expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

PTEN (F-1): sc-393186 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PTEN gene expression knockdown using RT-PCR Primer: PTEN (h2)-PR: sc-44272-PR (20 μ l, 484 bp). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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