SANTA CRUZ BIOTECHNOLOGY, INC.

DMBT1 siRNA (m): sc-35197

BACKGROUND

As human tumors progress to advanced stages, one genetic alteration that occurs at high frequency is a loss of heterozygosity (LOH) at chromosome 10. Mapping of homozygous deletions on this chromosome led to the isolation of the PTEN (also designated MMAC1 and TEP1), DMBT1 (for deleted in malignant brain tumors 1) and LGI1 (for leucine-rich gene-glioma inactivated 1) candidate tumor suppressor genes. The PTEN 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. Reduced levels of DMBT1 mRNA have been noted in gastrointestinal and esophageal cancers as well as in gliomas. LGI1, which is highly specific for neural tissues, shares homology with several transmembrane and extracellular proteins that function as receptors and adhesion proteins.

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. Mollenhauer,J., et al. 1997. DMBT1, a new member of the SRCR superfamily, on chromosome 10q25.3-q26.1 is deleted in malignant brain tumours. Nat. Genet. 17: 32-39.
6. Chernova, O.B., et al. 1998. A novel gene, LGI1, from 10q24 is rearranged and downregulated in malignant brain tumors. Oncogene 17: 2873-2881.
7. Mori, M., et al. 1999. Lack of DMBT1 expression in oesophageal, gastric and colon cancers. Br. J. Cancer 79: 211-213.
8. Munoz, J. and Castresana, J.S. 2006. Silencing of DMBT1 in neuroblastoma cell lines is not due to methylation of CCWGG motifs on its promoter. Neoplasma 53: 15-18.

CHROMOSOMAL LOCATION

Genetic locus: Dmbt1 (mouse) mapping to 7 F3.

PRODUCT

DMBT1 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 DMBT1 shRNA Plasmid (m): sc-35197-SH and DMBT1 shRNA (m) Lentiviral Particles: sc-35197-V as alternate gene silencing products.

For independent verification of DMBT1 (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-35197A, sc-35197B and sc-35197C.

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

DMBT1 siRNA (m) is recommended for the inhibition of DMBT1 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

DMBT1 (G-4): sc-514566 is recommended as a control antibody for monitoring of DMBT1 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 DMBT1 gene expression knockdown using RT-PCR Primer: DMBT1 (m)-PR: sc-35197-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.