

MGMT siRNA (h): sc-35927

BACKGROUND

MGMT (O^6 -methylguanine-DNA methyltransferase) is transcriptionally activated in response to DNA damage and functions to repair mutagenic and cytotoxic O^6 -alkylguanine lesions caused by carcinogens or cytostatic drugs. MGMT induction by ionising radiation does not occur in p53-deficient mice, suggesting that MGMT induction may require p53. Similarly, MGMT mRNA and protein were shown to be inducible by ionising radiation only in cell lines that express functional p53, and not in cell lines that do not express wild type p53. In contrast, in a study of oral cancer cell lines, high MGMT activity was associated with the presence of mutant p53. Similarly, MGMT activity was significantly lower in ovarian tumors with wildtype p53 than in tumors with mutant p53, supporting the view that wild type p53 down-regulates the basal MGMT promoter.

CHROMOSOMAL LOCATION

Genetic locus: MGMT (human) mapping to 10q26.3.

PRODUCT

MGMT 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MGMT shRNA Plasmid (h): sc-35927-SH and MGMT shRNA (h) Lentiviral Particles: sc-35927-V as alternate gene silencing products.

For independent verification of MGMT (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-35927A, sc-35927B and sc-35927C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

MGMT siRNA (h) is recommended for the inhibition of MGMT 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 μ M in 66 μ l. 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

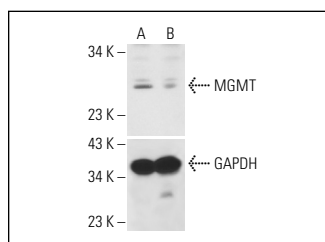
MGMT (E-1): sc-166528 is recommended as a control antibody for monitoring of MGMT gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 MGMT gene expression knockdown using RT-PCR Primer: MGMT (h)-PR: sc-35927-PR (20 μ l, 428 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

DATA



MGMT siRNA (h): sc-35927. Western blot analysis of MGMT expression in non-transfected control (A) and MGMT siRNA transfected (B) HeLa cells. Blot probed with MGMT (C-20): sc-8825. GAPDH (FL-335): sc-25778 used as specificity and loading control.

SELECT PRODUCT CITATIONS

- Zheng, M., et al. 2008. Interleukin-24 overcomes temozolomide resistance and enhances cell death by down-regulation of O^6 -methylguanine-DNA methyltransferase in human melanoma cells. *Mol. Cancer Ther.* 7: 3842-3851.
- Thanasupawat, T., et al. 2017. Dovitinib enhances temozolomide efficacy in glioblastoma cells. *Mol. Oncol.* 11: 1078-1098.
- Yu, Z., et al. 2018. Inhibition of NF κ B results in anti-glioma activity and reduces temozolomide-induced chemoresistance by down-regulating MGMT gene expression. *Cancer Lett.* 428: 77-89.

RESEARCH USE

For research use only, not for use in diagnostic procedures.