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MUTYH shRNA (m) Lentiviral Particles: sc-45816-V



The Power to Overtion

BACKGROUND

MUTYH (mutY homolog (*E. coli*)) is a DNA glycosylase mismatch repair enzyme that, in conjunction with mutM (OGG1), cleaves adenine residues paired with either oxidized (8-hydroxyguanines) or non-modified guanines in order to correct A/G and A/C mismatches. Repair of most modified and mispaired bases in the genome is initiated by DNA glycosylases, which bind and cleave N-glycosyl bonds to initiate base excision repair. MUTYH is crucial for the avoidance of mutations resulting from oxidative DNA damage. Multiple N-terminal splice variants of MUTYH exist in mammalian cells. Increasing levels of MUTYH in A549 cells exposed to oxygen and infrared radiation leads to improvements in cell survival. Biallelic MUTYH germ-line mutations predispose humans to colorectal adenomas and carcinomas. MUTYH is abundant in neurons where mitochondrial genomes exposed to reactive oxygen species (ROS) that damage DNA must maintain integrity over the entire mammalian life span.

REFERENCES

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- Englander, E.W., et al. 2002. Rat MYH, a glycosylase for repair of oxidatively damaged DNA, has brain-specific isoforms that localize to neuronal mitochondria. J. Neurochem. 83: 1471-1480.
- Halford, S.E., et al. 2003. Germline mutations but not somatic changes at the MYH locus contribute to the pathogenesis of unselected colorectal cancers. Am. J. Pathol. 162: 1545-1548.
- Lee, H.M., et al. 2004. Developmental changes in expression and subcellular localization of the DNA repair glycosylase, MYH, in the rat brain. J. Neurochem. 88: 394-400.
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CHROMOSOMAL LOCATION

Genetic locus: Mutyh (mouse) mapping to 4 D1.

PRODUCT

MUTYH shRNA (m) 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 x 10⁶ 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 MUTYH siRNA (m): sc-45816 and MUTYH shRNA Plasmid (m): sc-45816-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

MUTYH shRNA (m) Lentiviral Particles is recommended for the inhibition of MUTYH expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 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

MUTYH (C-6): sc-374571 is recommended as a control antibody for monitoring of MUTYH 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) Immunofluo-rescence: 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 MUTYH gene expression knockdown using RT-PCR Primer: MUTYH (m)-PR: sc-45816-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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

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