SANTA CRUZ BIOTECHNOLOGY, INC.

MUTYH siRNA (h): sc-37407

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 im-provements 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

1. Hayashi, H., et al. 2002. Replication-associated repair of adenine: 8-oxoguanine mispairs by MYH. Curr. Biol. 12: 335-339.
2. 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.
3. 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.
4. 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.
5. Tao, H., et al. 2004. A novel splice-site variant of the base excision repair gene MYH is associated with production of an aberrant mRNA transcript encoding a truncated MYH protein not localized in the nucleus. Carcinogenesis 25: 1859-1866.
6. Kim, C.J., et al. 2004. Genetic alterations of the MYH gene in gastric cancer. Oncogene 23: 6820-6822.

CHROMOSOMAL LOCATION

Genetic locus: MUTYH (human) mapping to 1p34.1.

PRODUCT

MUTYH 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MUTYH shRNA Plasmid (h): sc-37407-SH and MUTYH shRNA (h) Lentiviral Particles: sc-37407-V as alternate gene silencing products.

For independent verification of MUTYH (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-37407A, sc-37407B and sc-37407C.

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

MUTYH siRNA (h) is recommended for the inhibition of MUTYH 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 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

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 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 MUTYH gene expression knockdown using RT-PCR Primer: MUTYH (h)-PR: sc-37407-PR (20 µl, 589 bp). 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.