SANTA CRUZ BIOTECHNOLOGY, INC.

MLH3 siRNA (h): sc-37408

BACKGROUND

DNA mismatch repair (MMR) is essential for maintaining the integrity of the genome during replication. This process is highly conserved across bacterial and eukaryotic systems, as many of the genes expressed in bacteria are closely related to the yeast and mammalian homologs. In bacteria two proteins, MutS and MutL, form homodimeric complexes that are responsible for recognizing and facilitating MMR. Human homologs of these proteins include MSH2 and MSH3 (MutS homolog 2 and 3), and the corresponding human homologs of MutL are MLH1, PMS1, PMS2 and MLH3. MSH2 and MSH3 form heterodimers that cooperatively mediate MMR. MLH3 preferentially dimerizes with MLH1 to repair DNA mismatches and restore the stability to the genome. Mutations in the genes encoding MSH2 and MLH1 induce microsatellite instablitiy of the DNA. These mutations are associated with the occurrence of hereditary nonpolyposis colorectal cancer (HNPCC) and are a common feature in the progression of many other cancers.

REFERENCES

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2. Palombo, F., et al. 1994. Mismatch repair and cancer. Nature 367: 417.
3. Watanabe, A, et al. 1996. Genomic organization and expression of the human MSH3 gene. Genomics 31: 311-318.
4. Prolla, T.A., et al. 1998. Tumour susceptibility and spontaneous mutation in mice deficient in Mlh1, Pms1 and Pms2 DNA mismatch repair. Nat. Genet. 18: 276-279.
5. Yao, X., et al. 1999. Different mutator phenotypes in Mlh1- versus Pms2-deficient mice. Proc. Natl. Acad. Sci. USA 96: 6850-6855.
6. Kolodner, R.D., et al. 1999. Eukaryotic DNA mismatch repair. Curr. Opin. Genet. Dev. 9: 89-96.
7. Harfe, B.D., et al. 2000. Discrete *in vivo* roles for the MutL homologs MLH2p and MLH3p in the removal of frameshift intermediates in budding yeast. Curr. Biol. 10: 145-148.
8. Lipkin, S.M., et al. 2000. MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. Nat. Genet. 24: 27-35.

CHROMOSOMAL LOCATION

Genetic locus: MLH3 (human) mapping to 14q24.3.

PRODUCT

MLH3 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 MLH3 shRNA Plasmid (h): sc-37408-SH and MLH3 shRNA (h) Lentiviral Particles: sc-37408-V as alternate gene silencing products.

For independent verification of MLH3 (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-37408A, sc-37408B and sc-37408C.

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

MLH3 siRNA (h) is recommended for the inhibition of MLH3 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

MLH3 (H-2): sc-25313 is recommended as a control antibody for monitoring of MLH3 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 MLH3 gene expression knockdown using RT-PCR Primer: MLH3 (h)-PR: sc-37408-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.