SANTA CRUZ BIOTECHNOLOGY, INC.

MLH1 siRNA (m): sc-35944

BACKGROUND

DNA-mismatch repair (MMR) is an essential process in maintaining genetic stability. Lack of a functional DNA-mismatch repair pathway is a common characteristic of several different types of human cancers, either due to an MMR gene mutation or promoter methylation gene silencing. MLH1 is an integral part of the protein complex responsible for mismatch repair that is expressed in lymphocytes, heart, colon, breast, lung, spleen, testis, prostate, thyroid and gall bladder and is methylated in several ovarian tumors. Loss of MLH1 protein expression is associated with a mutated phenotype, microsatellite instability and a predisposition to cancer. In hereditary nonpolyposis colorectal cancer (HNPCC), an autosomal dominant inherited cancer syndrome that signifies a high risk of colorectal and various other types of cancer, the MLH1 gene exhibits a pathogenic mutation. Certain cancer cell lines, including leukemia CCRF-CEM, colon HCT 116 and KM12, and ovarian cancers SK-OV-3 and IGROV-1, show complete deficiency of MLH1, while MLH1 is expressed in 60% of melanomas, 70% of noninvasive squamous cell carcinomas and 30% of invasive squamous cell carcinomas.

REFERENCES

1. Taverna, P., et al. 2000. Characterization of MLH1 and MSH2 DNA mismatch repair proteins in cell lines of the NCI anticancer drug screen. Cancer Chemother. Pharmacol. 46: 507-516.
2. Jarvinen, H.J., et al. 2000. Surveillance on mutation carriers of DNA mismatch repair genes. Ann. Chir. Gynaecol. 89: 207-210.
3. Korabiowska, M., et al. 2000. Analysis of the DNA mismatch repair proteins expression in malignant melanomas. Anticancer Res. 20: 4499-4505.
4. Giarnieri, E., et al. 2000. MSH2, MLH1, Fhit, p53, Bcl-2, and Bax expression in invasive and *in situ* squamous cell carcinoma of the uterine cervix. Clin. Cancer Res. 6: 3600-3606.
5. Korabiowska, M., et al. 2001. Relation between DNA ploidy status and the expression of the DNA-mismatch repair genes MLH1 and MSH2 in cytological specimens of melanoma lymph node and liver metastases. Diagn. Cytopathol. 24: 157-162.
6. Hardman, R.A., et al. 2001. Involvement of mammalian MLH1 in the apoptotic response to peroxide-induced oxidative stress. Cancer Res. 61: 1392-1397.

CHROMOSOMAL LOCATION

Genetic locus: Mlh1 (mouse) mapping to 9 F3.

PRODUCT

MLH1 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 MLH1 shRNA Plasmid (m): sc-35944-SH and MLH1 shRNA (m) Lentiviral Particles: sc-35944-V as alternate gene silencing products.

For independent verification of MLH1 (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-35944A, sc-35944B and sc-35944C.

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

MLH1 siRNA (m) is recommended for the inhibition of MLH1 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

MLH1 (B-12): sc-271978 is recommended as a control antibody for monitoring of MLH1 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 MLH1 gene expression knockdown using RT-PCR Primer: MLH1 (m)-PR: sc-35944-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.