SANTA CRUZ BIOTECHNOLOGY, INC.

MUS81 siRNA (h): sc-40751

BACKGROUND

Together, DNA repair and checkpoint responses ensure the integrity of the genome. Coordination of cell cycle checkpoints and DNA repair are especially important following genotoxic radiation or chemotherapy, during which unusually high loads of DNA damage are sustained. MUS81 encodes a helix-hairpin-helix protein involved in the response to UV- and methylation-induced DNA damage in *Saccharomyces cerevisiae*. MUS81 is important for replicational stress tolerance in both budding and fission yeast. Specifically, MUS81 associates with Eme1 to form an endonuclease that can process stalled replication forks before they have regressed to form a Holliday junction (2-4). MUS81 associated endonuclease resolves Holliday junctions into linear duplexes by cutting across the junction exclusively on strands of like polarity. In addition, MUS81 protein abundance increases in cells following exposure to agents that block DNA replication. MUS81 is involved in the recruitment of Cds1 to aberrant DNA structures where Cds1 modulates the activity of damage tolerance enzymes. The gene encoding human MUS81 maps to chromosome 11q13.1 and encodes a 551 amino acid protein.

REFERENCES

1. Interthal, H. and Heyer, W.D. 2000. MUS81 encodes a novel helix-hairpin-helix protein involved in the response to UV- and methylation-induced DNA damage in *Saccharomyces cerevisiae*. Mol. Gen. Genet. 263: 812-827.
2. Boddy, M.N., Lopez-Girona, A., Shanahan, P., Interthal, H., Heyer, W.D. and Russell, P. 2000. Damage tolerance protein MUS81 associates with the FHA1 domain of checkpoint kinase Cds1. Mol. Cell. Biol. 20: 8758-8766.
3. Chen, X.B., Melchionna, R., Denis, C.M., Gaillard, P.H., Blasina, A., Van de Weyer, I., Boddy, M.N., Russell, P., Vialard, J. and McGowan, C.H. 2001. Human Mus81-associated endonuclease cleaves Holliday junctions *in vitro*. Mol. Cell 8: 1117-1127.
4. Doe, C.L., Ahn, J.S., Dixon, J. and Whitby, M.C. 2002. MUS81-eme1 and rqh1 involvement in processing stalled and collapsed replication forks. J. Biol. Chem. 277: 32753-32759.
5. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606591. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: MUS81 (human) mapping to 11q13.1.

PRODUCT

MUS81 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 MUS81 shRNA Plasmid (h): sc-40751-SH and MUS81 shRNA (h) Lentiviral Particles: sc-40751-V as alternate gene silencing products.

For independent verification of MUS81 (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-40751A, sc-40751B and sc-40751C.

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

MUS81 shRNA Plasmid (h) is recommended for the inhibition of MUS81 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

MUS81 (B-12): sc-376661 is recommended as a control antibody for monitoring of MUS81 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 MUS81 gene expression knockdown using RT-PCR Primer: MUS81 (h)-PR: sc-40751-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.