SANTA CRUZ BIOTECHNOLOGY, INC.

DNA2L siRNA (m): sc-143074

BACKGROUND

The replication of lagging strand DNA requires several enzymatic steps that eventually lead to the generation of Okazaki fragments; small DNA strands that are attached by DNA Ligase. DNA2L (DNA2-like helicase), also known as DNA2, is a 1,060 amino acid human homolog of yeast DNA2, a helicase/nuclease that is essential for genome stability and DNA metabolism. In yeast, DNA2 acts as a DNA-dependent ATPase that unwinds duplex DNA, thereby producing single-standed DNA that serves as a template for DNA replication. In humans, DNA2L functions as a helicase that is involved in DNA replication, DNA repair and chromatin dynamics. Specifically, DNA2L is thought to play a role in the maturation and elongation of Okazaki fragments, thereby facilitating replication of the lagging strand. Mutations in the gene encoding DNA2L are lethal, suggesting that proper DNA2L function is crucial for cell viability. Three isoforms of DNA2L exist due to alternative splicing events.

REFERENCES

1. Eki, T., Okumura, K., Shiratori, A., Abe, M., Nogami, M., Taguchi, H., Shibata, T., Murakami, Y. and Hanaoka, F. 1996. Assignment of the closest human homologue (DNA2L:KIAA0083) of the yeast Dna2 helicase gene to chromosome band 10q21.3-q22.1. Genomics 37: 408-410.
2. Bae, S.H., Bae, K.H., Kim, J.A. and Seo, Y.S. 2001. RPA governs endonuclease switching during processing of Okazaki fragments in eukaryotes. Nature 412: 456-461.
3. Rossi, M.L. and Bambara, R.A. 2006. Reconstituted Okazaki fragment processing indicates two pathways of primer removal. J. Biol. Chem. 281: 26051-26061.
4. Masuda-Sasa, T., Polaczek, P. and Campbell, J.L. 2006. Single strand annealing and ATP-independent strand exchange activities of yeast and human DNA2: possible role in Okazaki fragment maturation. J. Biol. Chem. 281: 38555-38564.
5. Stewart, J.A., Campbell, J.L. and Bambara, R.A. 2006. Flap endonuclease disengages Dna2 helicase/nuclease from Okazaki fragment flaps. J. Biol. Chem. 281: 38565-38572.

CHROMOSOMAL LOCATION

Genetic locus: Dna2 (mouse) mapping to 10 B4.

PRODUCT

DNA2L 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 DNA2L shRNA Plasmid (m): sc-143074-SH and DNA2L shRNA (m) Lentiviral Particles: sc-143074-V as alternate gene silencing products.

For independent verification of DNA2L (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-143074A, sc-143074B and sc-143074C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

DNA2L siRNA (m) is recommended for the inhibition of DNA2L 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

DNA2L (A-3): sc-393323 is recommended as a control antibody for monitoring of DNA2L 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 DNA2L gene expression knockdown using RT-PCR Primer: DNA2L (m)-PR: sc-143074-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.