SANTA CRUZ BIOTECHNOLOGY, INC.

DNA Ligase III siRNA (h): sc-72079

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

DNA ligase is a type of ligase that can link together DNA strands that have double-strand breaks. DNA ligase functions in both DNA repair and DNA replication. It is utilized in molecular biology laboratories for recombination experiments. In mammals, the four specific types of DNA ligase are known as DNA Ligase I, II, III and IV. DNA Ligase I ligates Okazaki fragments during lagging strand DNA replication and some recombinant fragments. DNA Ligase II is an alternatively spliced form of DNA Ligase III found in non-dividing cells. DNA Ligase III complexes with the DNA repair protein XRCC1 to function in sealing base excision mutations and recombinant fragments. DNA Ligase IV complexes with XRCC4 and catalyzes the final step in the non-homologous end joining DNA double-strand break repair pathway.

REFERENCES

1. Lehman, I.R. 1974. DNA ligase: structure, mechanism, and function. Science 186: 790-797.
2. Caldecott, K.W., et al. 1994. An interaction between the mammalian DNA repair protein XRCC1 and DNA Ligase III. Mol. Cell. Biol. 14: 68-76.
3. Wei, Y.F., et al. 1995. Molecular cloning and expression of human cDNAs encoding a novel DNA Ligase IV and DNA Ligase III, an enzyme active in DNA repair and recombination. Mol. Cell. Biol. 15: 3206-3216.
4. Chen, J., et al. 1995. Mammalian DNA Ligase III: molecular cloning, chromosomal localization, and expression in spermatocytes undergoing meiotic recombination. Mol. Cell. Biol. 15: 5412-5422.
5. Caldecott, K.W., et al. 1997. XRCC1 polypeptide interacts with DNA polymerase β and possibly poly (ADP-ribose) polymerase, and DNA Ligase III is a novel molecular "nick-sensor" *in vitro*. Nucleic Acids Res. 24: 4387-4394.
6. Grawunder, U., et al. 1997. Activity of DNA Ligase IV stimulated by complex formation with XRCC4 protein in mammalian cells. Nature 388: 492-495.

CHROMOSOMAL LOCATION

Genetic locus: LIG3 (human) mapping to 17q12.

PRODUCT

DNA Ligase III 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 DNA Ligase III shRNA Plasmid (h): sc-72079-SH and DNA Ligase III shRNA (h) Lentiviral Particles: sc-72079-V as alternate gene silencing products.

For independent verification of DNA Ligase III (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-72079A, sc-72079B and sc-72079C.

PROTOCOLS

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

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

DNA Ligase III siRNA (h) is recommended for the inhibition of DNA Ligase III 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

DNA Ligase III (E-7): sc-390922 is recommended as a control antibody for monitoring of DNA Ligase III 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 DNA Ligase III gene expression knockdown using RT-PCR Primer: DNA Ligase III (h)-PR: sc-72079-PR (20 µl, 472 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.