SANTA CRUZ BIOTECHNOLOGY, INC.

DNA Ligase IV siRNA (h): sc-37394

BACKGROUND

The X-ray repair cross-complementing protein XRCC4 and DNA Ligase IV are essential for repairing double-strand breaks in DNA. These proteins form a critical complex consisting of two molecules of each protein that preferentially bind DNA with nicks or broken ends. As an obligate accessory molecule, XRCC4 binds to DNA Ligase IV and enhances its joining activity. The XRCC4/DNA Ligase IV complex is also involved in V(D)J recombination. V(D)J recombination occurs in normal development of the adaptive immune system and involves the formation of a double-strand break intermediate. Deletions of either DNA Ligase IV or XRCC4 inhibit the completion of V(D)J recombination, resulting in a high incidence of apoptosis in the developing nervous system and a block in B and T cell maturation.

REFERENCES

1. Modesti, M., et al. 1999. DNA binding of XRCC4 protein is associated with V(D)J recombination but not with stimulation of DNA Ligase IV activity. EMBO J. 18: 2008-2018.
2. Bryans, M., et al. 1999. Absence of DNA Ligase IV protein in XR-1 cells: evidence for stabilization by XRCC4. Mutat. Res. 433: 53-58.
3. Chen, L., et al. 2000. Interactions of the DNA Ligase IV/XRCC4 complex with DNA ends and the DNA-dependent protein kinase. J. Biol. Chem. 275: 26196-26205.
4. Lee, K.J., et al. 2000. DNA Ligase IV and XRCC4 form a stable mixed tetramer that functions synergistically with other repair factors in a cell-free end-joining system. J. Biol. Chem. 275: 34787-34796.
5. Junop, M.S., et al. 2000. Crystal structure of the XRCC4 DNA repair protein and implications for end-joining. EMBO J. 19: 5962-5970.
6. Moshous, D., et al. 2000. New gene involved in DNA double-strand break repair and V(D)J recombination is located on human chromosome 10p. Hum. Mol. Genet. 9: 583-588.
7. Muylaert, I., et al. 2007. Knockdown of DNA Ligase IV/XRCC4 by RNA interference inhibits herpes simplex virus type 1 DNA replication. J. Biol. Chem. 282: 10865-10872.

CHROMOSOMAL LOCATION

Genetic locus: LIG4 (human) mapping to 13q33.3.

PRODUCT

DNA Ligase IV 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 IV shRNA Plasmid (h): sc-37394-SH and DNA Ligase IV shRNA (h) Lentiviral Particles: sc-37394-V as alternate gene silencing products.

For independent verification of DNA Ligase IV (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-37394A, sc-37394B and sc-37394C.

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 IV siRNA (h) is recommended for the inhibition of DNA Ligase IV 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 IV (D-8): sc-271299 is recommended as a control antibody for monitoring of DNA Ligase IV 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 IV gene expression knockdown using RT-PCR Primer: DNA Ligase IV (h)-PR: sc-37394-PR (20 µl, 545 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Wang, W.Y., et al. 2013. Interaction of FUS and HDAC1 regulates DNA damage response and repair in neurons. Nat. Neurosci. 16: 1383-1391.

RESEARCH USE

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