SANTA CRUZ BIOTECHNOLOGY, INC.

XLF siRNA (m): sc-76937

BACKGROUND

XLF (XRCC4-like factor), also known as non-homologous end-joining factor 1 (NHEJ1) or cernunnos, is a 295 amino acid protein belonging to the XLF family. There are two main repair pathways for DNA double-strand breaks: homologous recombination (HR) and non-homologous end-joining (NHEJ). In the latter pathway, the Ku-70/Ku-86 heterodimer binds the DNA ends together and the DNA-PK catalytic subunits are recruited. Then the DNA ends are processed by DNA processing enzymes, such as Artemis. The binding is finalized through DNA Ligase IV, which acts in a complex with XRCC4 and XLF to stabilize the repair. Thus, it is believed that XLF interacts with DNA Ligase IV and XRCC4 to constitute the enzymatic core of the NHEJ machinery. Two named isoforms of XLF exist as a result of alternative splicing events.

REFERENCES

1. Revy, P., et al. 2006. Cernunnos-XLF, a recently identified non-homologous end-joining factor required for the development of the immune system. Curr. Opin. Allergy Clin. Immunol. 6: 416-420.
2. Drouet, J., et al. 2006. Interplay between Ku, Artemis, and the DNA-dependent protein kinase catalytic subunit at DNA ends. J. Biol. Chem. 281: 27784-27793.
3. Hentges, P., et al. 2006. Evolutionary and functional conservation of the DNA non-homologous end-joining protein, XLF/Cernunnos. J. Biol. Chem. 281: 37517-37526.
4. Windhofer, F., et al. 2007. Low levels of DNA ligases III and IV sufficient for effective NHEJ. J. Cell. Physiol. 213: 475-483.
5. Zha, S., et al. 2007. Defective DNA repair and increased genomic instability in Cernunnos-XLF-deficient murine ES cells. Proc. Natl. Acad. Sci. USA 104: 4518-4523.
6. Tsai, C.J., et al. 2007. Cernunnos/XLF promotes the ligation of mismatched and noncohesive DNA ends. Proc. Natl. Acad. Sci. USA 104: 7851-7856.
7. Mahaney, B.L., et al. 2009. Repair of ionizing radiation-induced DNA double-strand breaks by non-homologous end-joining. Biochem. J. 417: 639-650.
8. Malivert, L., et al. 2009. The C-terminal domain of Cernunnos/XLF is dispensable for DNA repair *in vivo*. Mol. Cell. Biol. 29: 1116-1122.

CHROMOSOMAL LOCATION

Genetic locus: Nhej1 (mouse) mapping to 1 C3.

PRODUCT

XLF 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 XLF shRNA Plasmid (m): sc-76937-SH and XLF shRNA (m) Lentiviral Particles: sc-76937-V as alternate gene silencing products.

For independent verification of XLF (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-76937A, sc-76937B and sc-76937C.

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

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

XLF (D-1): sc-166488 is recommended as a control antibody for monitoring of XLF 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 XLF gene expression knockdown using RT-PCR Primer: XLF (m)-PR: sc-76937-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.