

XPF siRNA (m): sc-36856

BACKGROUND

Xeroderma pigmentosum (XP) is an autosomal recessive disorder characterized by a genetic predisposition to sunlight-induced skin cancer, and it is commonly due to deficiencies in DNA repair enzymes. The most frequent mutations are found in the XP genes from group A through G and group V, which encode for nucleotide excision repair proteins. XPF, which is also designated ERCC4 or ERCC11, is a protein that associates directly with the excision repair cross-complementing 1 (ERCC1) factor. ERCC1, a functional homolog of Rad10 in *S. cerevisiae*, is a component of a structure-specific endonuclease that is responsible for 5' incisions during DNA repair. The ERCC1-XPF endonuclease preferentially cleaves one strand of DNA between duplex and single-stranded regions near borders of the stem-loop structure, thereby contributing to the initial steps of the nucleotide excision repair process.

REFERENCES

1. van Duin, M., et al. 1986. Molecular characterization of the human excision repair gene ERCC1: cDNA cloning and amino acid homology with the yeast DNA repair gene Rad10. *Cell* 44: 913-923.
2. Li, L., et al. 1995. Mutations in XPA that prevent association with ERCC1 are defective in nucleotide excision repair. *Mol. Cell. Biol.* 15: 1993-1998.
3. Tateishi, S., et al. 1995. Separation of protein factors that correct the defects in the seven complementation groups of xeroderma pigmentosum cells. *J. Biochem.* 118: 819-824.
4. Aboussekha, A., et al. 1995. Mammalian DNA nucleotide excision repair reconstituted with purified protein components. *Cell* 80: 859-868.
5. Sijbers, A.M., et al. 1996. Xeroderma pigmentosum group F caused by a defect in a structure-specific DNA repair endonuclease. *Cell* 86: 811-822.
6. Wakasugi, M. and Sancar, A. 1999. Order of assembly of human DNA repair excision nuclease. *J. Biol. Chem.* 274: 18759-18768.
7. Houtsmuller, A.B., et al. 1999. Action of DNA repair endonuclease ERCC1/XPF in living cells. *Science* 284: 958-961.

CHROMOSOMAL LOCATION

Genetic locus: *Erc4* (mouse) mapping to 16 A1.

PRODUCT

XPF 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see XPF shRNA Plasmid (m): sc-36856-SH and XPF shRNA (m) Lentiviral Particles: sc-36856-V as alternate gene silencing products.

For independent verification of XPF (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-36856A, sc-36856B and sc-36856C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

XPF siRNA (m) is recommended for the inhibition of XPF 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 μ M in 66 μ l. 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

XPF (F-11): sc-398032 is recommended as a control antibody for monitoring of XPF 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 XPF gene expression knockdown using RT-PCR Primer: XPF (m)-PR: sc-36856-PR (20 μ l). 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.