SANTA CRUZ BIOTECHNOLOGY, INC.

XPA siRNA (m): sc-36854

BACKGROUND

Xeroderma pigmentosum (XP) is an autosomal recessive disorder characterized by a genetic predisposition to sunlight-induced skin cancer due to deficiencies in the DNA repair enzymes. The most frequent mutations are found in the XP genes of group A through G and group V, which encode nucleotide excision repair proteins. Nucleotide excision repair (NER) is the normal cellular response to DNA damage induced by UV irradiation and is disrupted in patients with XP. Xeroderma pigmentosum group A (XPA) is an essential NER factor that coordinates the collection of a preincision complex during the processing of DNA damage. XPA may also have a role in the repair of oxidized DNA bases. XPA is sensitive not only to the structure of the DNA double helix, but also to bulky groups incorporated into DNA. XPA forms a homodimer in the absence of DNA, but binds to DNA in both monomeric and dimeric forms. The dimerically bound XPA is much more efficient, so cells probably regulate XPA activity in a concentration-dependent manner. XPA deficient organisms cannot repair UV-induced DNA damage and thus acquire skin cancers by UV irradiation very easily.

REFERENCES

1. 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.
2. Nakane, H., et al. 1995. High incidence of ultraviolet-B- or chemical-carcinogen-induced skin tumours in mice lacking the xeroderma pigmentosum group A gene. Nature 377: 165-168.
3. Kuraoka, I., et al. 1996. Identification of a damaged-DNA binding domain of the XPA protein. Mut. Res. 362: 87-95.
4. Riou, L., et al. 1999. The relative expression of mutated XPB genes results in xeroderma pigmentosum/Cockayne's syndrome or trichothiodystrophy cellular phenotypes. Hum. Mol. Genet. 8: 1125-1133.
5. Horio, T., et al. 2001. Photobiologic and photoimmunologic characteristics of XPA gene-deficient mice. J. Investig. Dermatol. Symp. Proc. 6: 58-63.
6. Patrick, S.M., et al. 2002. Xeroderma pigmentosum complementation group A protein (XPA) modulates RPA-DNA interactions via enhanced complex stability and inhibition of strand separation activity. J. Biol. Chem. 277: 16096-16101.

CHROMOSOMAL LOCATION

Genetic locus: Xpa (mouse) mapping to 4 B1.

PRODUCT

XPA 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 XPA shRNA Plasmid (m): sc-36854-SH and XPA shRNA (m) Lentiviral Particles: sc-36854-V as alternate gene silencing products.

For independent verification of XPA (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-36854A, sc-36854B and sc-36854C.

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

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

XPA (B-1): sc-28353 is recommended as a control antibody for monitoring of XPA 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 XPA gene expression knockdown using RT-PCR Primer: XPA (m)-PR: sc-36854-PR (20 µl, 428 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.

PROTOCOLS

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