SANTA CRUZ BIOTECHNOLOGY, INC.

XPG siRNA (h): sc-36857

BACKGROUND

Seven complementation groups (A-G) of xeroderma pigmentosum have been described. The xeroderma pigmentosum group A protein, XPA, is a zinc metalloprotein which preferentially binds to DNA damaged by ultraviolet (UV) radiation and chemical carcinogens. XPA is a DNA repair enzyme that has been shown to be required for the incision step of nucleotide excision repair. XPG (also designated ERCC5) is an endonuclease that makes the 3' incision in DNA nucleotide excision repair. Mammalian XPG is similar in sequence to yeast RAD2. Conserved residues in the catalytic center of XPG are important for nuclease activity and function in nucleotide excision repair.

REFERENCES

1. Scherly, D., et al. 1993. Complementation of the DNA repair defect in xeroderma pigmentosum group G cells by a human cDNA related to yeast RAD2. Nature 363: 182-185.
2. Shiomi, T., et al. 1994. An ERCC5 gene with homology to yeast RAD2 is involved in group G xeroderma pigmentosum. Mutat. Res. 314: 167-175.
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. 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.
5. 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.
6. Kuraoka, I., et al. 1996. Identification of a damaged-DNA binding domain of the XPA protein. Mutat. Res. 362: 87-95.
7. Constantinou, A., et al. 1999. Conserved residues of human XPG protein important for nuclease activity and function in nucleotide excision repair. J. Biol. Chem. 274: 5637-5648.
8. Cappelli, E., et al. 1999. The DNA helicases acting in nucleotide excision repair, XPD, CSB and XPB, are not required for PCNA-dependent repair of abasic sites. Eur. J. Biochem. 259: 325-330.

CHROMOSOMAL LOCATION

Genetic locus: ERCC5 (human) mapping to 13q33.1.

PRODUCT

XPG 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 XPG shRNA Plasmid (h): sc-36857-SH and XPG shRNA (h) Lentiviral Particles: sc-36857-V as alternate gene silencing products.

For independent verification of XPG (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-36857A, sc-36857B and sc-36857C.

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

XPG siRNA (h) is recommended for the inhibition of XPG 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

XPG (8H7): sc-13563 is recommended as a control antibody for monitoring of XPG 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 XPG gene expression knockdown using RT-PCR Primer: XPG (h)-PR: sc-36857-PR (20 µl, 521 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.