SANTA CRUZ BIOTECHNOLOGY, INC.

RPA 32 kDa subunit siRNA (m): sc-38230

BACKGROUND

The single-stranded-DNA-binding proteins (SSBs) are essential for DNA function in prokaryotic and eukaryotic cells, mitochondria, phages and viruses. Replication protein A (RPA), a highly conserved eukaryotic protein, is a heterotrimeric SSB. RPA plays an important role in DNA replication, recombination and repair. The binding of human RPA (hRPA) to DNA involves molecular polarity in which initial hRPA binding occurs on the 5' side of a ssDNA substrate and then extends in the 3' direction to create a stably bound hRPA. RPA is a major damage-recognition protein involved in the early stages of nucleotide excision repair. It can also play a role in telomere maintenance. The C-terminus of RPA 32 can specfically intereact with the DNA repair enzyme UNG2 and repair factors XPA and Rad52, each of which functions in a different repair pathway. In addition, RPA 32 binds specifically to the SH2 domain of Stat3 *in vivo,* and overexpression of RPA 32 corresponds to the augmented growth factor-stimulated tyrosine phosphorylation and transcription activities of Stat3.

REFERENCES

1. Erdile, L.F., et al. 1990. The primary structure of the 32-kDa subunit of human replication protein A. J. Biol. Chem. 265: 3177-3182.
2. Erdile, L.F., et al. 1991. Characterization of a cDNA encoding the 70-kDa single-stranded DNA-binding subunit of human replication protein A and the role of the protein in DNA replication. J. Biol. Chem. 266: 12090-12098.
3. Bochkarev, A., et al. 1997. Structure of the single-stranded-DNA-binding domain of replication protein A bound to DNA. Nature 385: 176-181.
4. Kim, J., et al. 2000. Replication protein a 32 kDa subunit (RPA p32) binds the SH2 domain of STAT3 and regulates its transcriptional activity. Cell Biol. Int. 24: 467-473.
5. Mer, G., et al. 2000. Structural basis for the recognition of DNA repair proteins UNG2, XPA, and RAD52 by replication factor RPA. Cell 103: 449-456.
6. Iftode, C., et al. 2000. 5'→3' molecular polarity of human replication protein (hRPA) binding to pseudo-origin DNA substrates. Biochemistry 39: 11970-119981.

CHROMOSOMAL LOCATION

Genetic locus: Rpa2 (mouse) mapping to 4 D2.3.

PRODUCT

RPA 32 kDa subunit 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 RPA 32 kDa subunit shRNA Plasmid (m): sc-38230-SH and RPA 32 kDa subunit shRNA (m) Lentiviral Particles: sc-38230-V as alternate gene silencing products.

For independent verification of RPA 32 kDa subunit (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-38230A, sc-38230B and sc-38230C.

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

RPA 32 kDa subunit siRNA (m) is recommended for the inhibition of RPA 32 kDa subunit 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

RPA 32 kDa subunit (9H8): sc-56770 is recommended as a control antibody for monitoring of RPA 32 kDa subunit 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 RPA 32 kDa subunit gene expression knockdown using RT-PCR Primer: RPA 32 kDa subunit (m)-PR: sc-38230-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.