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SANTA CRUZ BIOTECHNOLOGY, INC.

RPA 14 kDa subunit siRNA (m): sc-45713

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 that is composed of three subunits, designated RPA 14 kDa (also known as RPA3), RPA 32 kDa and RPA 70 kDa. Together, these subunits play an important role in DNA replication, recombination and repair. RPA is one of the major damage-recognition structures involved in the early stage of nucleotide excision repair and may play a role in telomere maintenance. 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. The RPA 14 kDa subunit localizes to the nucleus and is the smallest component of the RPA complex, functioning with the other subunits to regulate various aspects of DNA metabolism.

REFERENCES

1. Umbricht, C.B., et al. 1993. Cloning, overexpression, and genomic mapping of the 14 kDa subunit of human replication protein A. J. Biol. Chem. 268: 6131-6138.
2. Umbricht, C.B., et al. 1994. High-resolution genomic mapping of the three human replication protein A genes (RPA1, RPA2, and RPA3). Genomics 20: 249-257.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 179837. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Zou, L., et al. 2003. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. Science 300: 1542-1548.
5. Dodson, G.E., et al. 2004. DNA replication defects, spontaneous DNA damage, and ATM-dependent checkpoint activation in replication protein A-deficient cells. J. Biol. Chem. 279: 34010-34014.

CHROMOSOMAL LOCATION

Genetic locus: Rpa3 (mouse) mapping to 6 A1.

PRODUCT

RPA 14 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 14 kDa subunit shRNA Plasmid (m): sc-45713-SH and RPA 14 kDa subunit shRNA (m) Lentiviral Particles: sc-45713-V as alternate gene silencing products.

For independent verification of RPA 14 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-45713A, sc-45713B and sc-45713C.

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 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 14 kDa subunit siRNA (m) is recommended for the inhibition of RPA 14 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 14 kDa subunit (A-2): sc-393891 is recommended as a control antibody for monitoring of RPA 14 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 14 kDa subunit gene expression knockdown using RT-PCR Primer: RPA 14 kDa subunit (m)-PR: sc-45713-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.