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RPA 14 kDa subunit shRNA (h) Lentiviral Particles: sc-45476-V

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

- 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.
- 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.
- 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/>
- Zou, L., et al. 2003. Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* 300: 1542-1548.

CHROMOSOMAL LOCATION

Genetic locus: RPA3 (human) mapping to 7p21.3.

PRODUCT

RPA 14 kDa subunit shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see RPA 14 kDa subunit siRNA (h): sc-45476 and RPA 14 kDa subunit shRNA Plasmid (h): sc-45476-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

RPA 14 kDa subunit shRNA (h) Lentiviral Particles is recommended for the inhibition of RPA 14 kDa subunit expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

RPA 14 kDa subunit (E-2): sc-271564 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 (h)-PR: sc-45476-PR (20 µl). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.