SANTA CRUZ BIOTECHNOLOGY, INC.

RFC4 siRNA (m): sc-36407

BACKGROUND

Replication factor C (RFC) is an essential DNA polymerase accessory protein that is required for numerous aspects of DNA metabolism including DNA replication, DNA repair, and telomere metabolism. RFC is a heteropentameric complex that recognizes a primer on a template DNA, binds to a primer terminus, and loads proliferating cell nuclear antigen (PCNA) onto DNA at primer-template junctions in an ATP-dependent reaction. All five of the RFC subunits share a set of related sequences (RFC boxes) that include nucleotide-binding consensus sequences. Four of the five RFC genes (RFC1, RFC2, RFC3, and RFC4) have consensus ATP-binding motifs. The small RFC proteins, RFC2, RFC3, RFC4 and RFC5, interact with Rad24, whereas the RFC1 subunit does not. Specifically, RFC4 plays a role in checkpoint regulation. RFC4 is a component of BASC (for BRCA1-associated genome surveillance complex) which serves as a sensor for abnormal DNA structures and/or as a regulator of the postreplication repair process. The human RFC4 gene maps to chromosome 3q27.3 and encodes the RFC4 subunit.

REFERENCES

1. Cullmann, G., et al. 1995. Characterization of the five replication factor C genes of *Saccharomyces cerevisiae*. Mol. Cell. Biol. 15: 4661-4671.
2. Beckwith, W.H., et al. 1998. Destabilized PCNA trimers suppress defective RFC1 proteins *in vivo* and *in vitro*. Biochemistry 37: 3711-3722.
3. Noskov, V.N., et al. 1998. The RFC2 gene, encoding the third-largest subunit of the replication factor C complex, is required for an S-phase checkpoint in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 18: 4914-4923.
4. Green, C.M., et al. 2000. A novel Rad24 checkpoint protein complex closely related to replication factor C. Curr. Biol. 10: 39-42.
5. Schmidt, S.L., et al. 2001. ATP utilization by yeast replication factor C. IV. RFC ATP-binding mutants show defects in DNA replication, DNA repair, and checkpoint regulation. J. Biol. Chem. 276: 34792-34800.
6. Krause, S.A., et al. 2001. Loss of cell cycle checkpoint control in *Drosophila* RFC4 mutants. Mol. Cell. Biol. 21: 5156-5168.
7. Online Mendelian Inheritance in Man, OMIM™. 2001. Johns Hopkins University, Baltimore, MD. MIM Number: 102577. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: Rfc4 (mouse) mapping to 16 B1.

PRODUCT

RFC4 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 RFC4 shRNA Plasmid (m): sc-36407-SH and RFC4 shRNA (m) Lentiviral Particles: sc-36407-V as alternate gene silencing products.

For independent verification of RFC4 (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-36407A, sc-36407B and sc-36407C.

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

RFC4 siRNA (m) is recommended for the inhibition of RFC4 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

RFC4 (C-9): sc-28301 is recommended as a control antibody for monitoring of RFC4 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 RFC4 gene expression knockdown using RT-PCR Primer: RFC4 (m)-PR: sc-36407-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.