



## Rif1 siRNA (h): sc-62944

### BACKGROUND

Rif1 (Rap1-interacting factor 1) is the 2,472 amino acid homolog of the yeast Rap1 protein and is highly expressed in testis, with increased expression in late G<sub>2</sub>/S phase of the cell cycle. Localized to midzone microtubules during anaphase and to condensed chromosomes during telophase, Rif1 is required for DNA-damage-induced, checkpoint-mediated cell cycle arrest during S phase. The yeast homolog of Rif1 functions by localizing to DNA-damaged foci and binding to uncapped telomeres, thereby inhibiting telomere elongation and slowing cell cycle progression. Human Rif1, unlike its yeast counterpart, does not participate in telomere maintenance or capping, but rather is thought to function at the microtubule midzone in a more global DNA damage response pathway. Rif1 may act by controlling transcription of telomere-related genes or by controlling resolution of twisted chromosomes by topoisomerase II (Topo II). Two isoforms of Rif1 exist due to alternative splicing events.

### REFERENCES

1. Li, B., et al. 2003. Rap1 affects the length and heterogeneity of human telomeres. *Mol. Biol. Cell* 14: 5060-5068.
2. Silverman, J., et al. 2004. Human Rif1, ortholog of a yeast telomeric protein, is regulated by ATM and 53BP1 and functions in the S-phase checkpoint. *Genes Dev.* 18: 2108-2119.
3. Banerjee, S., et al. 2004. Increased genome instability and telomere length in the *elg1*-deficient *Saccharomyces cerevisiae* mutant are regulated by S-phase checkpoints. *Eukaryotic Cell* 3: 1557-1566.
4. Xu, L., et al. 2004. Human Rif1 protein binds aberrant telomeres and aligns along anaphase midzone microtubules. *J. Cell Biol.* 167: 819-830.

### CHROMOSOMAL LOCATION

Genetic locus: RIF1 (human) mapping to 2q23.3.

### PRODUCT

Rif1 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Rif1 shRNA Plasmid (h): sc-62944-SH and Rif1 shRNA (h) Lentiviral Particles: sc-62944-V as alternate gene silencing products.

For independent verification of Rif1 (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-62944A, sc-62944B and sc-62944C.

### 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

Rif1 siRNA (h) is recommended for the inhibition of Rif1 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  $\mu$ M in 66  $\mu$ l. 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

Rif1 (B-3): sc-515573 is recommended as a control antibody for monitoring of Rif1 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-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 Rif1 gene expression knockdown using RT-PCR Primer: Rif1 (h)-PR: sc-62944-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

### SELECT PRODUCT CITATIONS

1. Alver, R.C., et al. 2017. Reversal of DDK-mediated MCM phosphorylation by Rif1-PP1 regulates replication initiation and replisome stability independently of ATR/Chk1. *Cell Rep.* 18: 2508-2520.

### RESEARCH USE

For research use only, not for use in diagnostic procedures.

### PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.