SANTA CRUZ BIOTECHNOLOGY, INC.

RAP1 siRNA (r): sc-270420

BACKGROUND

RAP1, also known as TERF2IP (telomeric repeat-binding factor 2-interacting protein 1) or DRIP5, is a 399 amino acid nuclear and cytoplasmic protein that contains one BRCT domain and one Myb-like domain. Belonging to the RAP1 family, RAP1 acts as both a regulator of telomere function and a regulator of transcription. While it does not bind DNA directly, RAP1 is recruited to telomeric double-stranded 5'-TTAGGG-3' repeats via its interaction with TRF2. RAP1 is required to negatively regulate telomere recombination and is essential for repressing homology-directed repair (HDR), which can affect telomere length. The gene that encodes RAP1 maps to human chromosome 16q23.1 and mouse chromosome 8 E1.

REFERENCES

1. Li, B., et al. 2000. Identification of human RAP1: implications for telomere evolution. Cell 101: 471-483.
2. Online Mendelian Inheritance in Man, OMIM™. 2000. Johns Hopkins University, Baltimore, MD. MIM Number: 605061. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
3. Hanaoka, S., et al. 2001. NMR structure of the hRAP1 Myb motif reveals a canonical three-helix bundle lacking the positive surface charge typical of Myb DNA-binding domains. J. Mol. Biol. 312: 167-175.
4. Tan, M., et al. 2003. The telomeric protein RAP1 is conserved in vertebrates and is expressed from a bidirectional promoter positioned between the RAP1 and KARS genes. Gene 323: 1-10.
5. Ye, J.Z., et al. 2004. TIN2 binds TRF1 and TRF2 simultaneously and stabilizes the TRF2 complex on telomeres. J. Biol. Chem. 279: 47264-47271.
6. Liu, D., et al. 2004. Telosome, a mammalian telomere-associated complex formed by multiple telomeric proteins. J. Biol. Chem. 279: 51338-51342.
7. Sarthy, J., et al. 2009. Human RAP1 inhibits non-homologous end joining at telomeres. EMBO J. 28: 3390-3399.
8. Martinez, P., et al. 2010. Mammalian RAP1 controls telomere function and gene expression through binding to telomeric and extratelomeric sites. Nat. Cell Biol. 12: 768-780.

CHROMOSOMAL LOCATION

Genetic locus: Terf2ip (rat) mapping to 19q12.

PRODUCT

RAP1 siRNA (r) 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 RAP1 shRNA Plasmid (r): sc-270420-SH and RAP1 shRNA (r) Lentiviral Particles: sc-270420-V as alternate gene silencing products.

For independent verification of RAP1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270420A, sc-270420B and sc-270420C.

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

RAP1 siRNA (r) is recommended for the inhibition of RAP1 expression in rat 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

RAP1 (4C8/1): sc-53434 is recommended as a control antibody for monitoring of RAP1 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 RAP1 gene expression knockdown using RT-PCR Primer: RAP1 (r)-PR: sc-270420-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.