SANTA CRUZ BIOTECHNOLOGY, INC.

PIF1 siRNA (m): sc-76135

BACKGROUND

PIF1 is a single-stranded (ss) DNA-dependent ATPase as well as a DNA helicase that unwinds DNA in a 5' to 3' direction with respect to the DNA strand on which it binds first. This protein is critical to DNA replication and telomere length maintenance in *Saccharomyces cerevisiae*. The PIF1 gene is highly conserved from yeast to humans. Mutations in the gene that encodes for PIF1 cause all telomeres to lengthen, suggesting that the PIF1 functions as a catalytic inhibitor of both new telomere formation and telomere elongation. Human PIF1 preferentially binds telomeric DNA where it inhibits telomerase activity. PIF1 specifically counteracts the RecQ homolog Sgs1 helicase activity, and degradation of PIF1 is mediated by the ubiquitin-26S proteasome pathway.

REFERENCES

1. Lahaye, A., et al. 1991. PIF1: a DNA helicase in yeast mitochondria. EMBO J. 10: 997-1007.
2. Lahaye, A., et al. 1994. PIF1 DNA helicase from *Saccharomyces cerevisiae*. Biochemical characterization of the enzyme. J. Biol. Chem. 268: 26155-26161.
3. Schulz, V.P., et al. 1994. The *Saccharomyces* PIF1 DNA helicase inhibits telomere elongation and *de novo* telomere formation. Cell 76: 145-155.
4. Zhou, J.Q., et al. 2002. *Schizosaccharomyces pombe* pfh1^{+} encodes an essential 5' to 3' DNA helicase that is a member of the PIF1 subfamily of DNA helicases. Mol. Biol. Cell 13: 2180-2191.
5. Boule, J.B., et al. 2006. Roles of PIF1-like helicases in the maintenance of genomic stability. Nucleic Acids Res. 34: 4147-4153.
6. Budd, M.E., et al. 2006. Evidence suggesting that PIF1 helicase functions in DNA replication with the Dna2 helicase/nuclease and DNA polymerase δ. Mol. Cell. Biol. 26: 2490-2500.
7. Wagner, M., et al. 2006. The absence of Top3 reveals an interaction between the Sgs1 and PIF1 DNA helicases in *Saccharomyces cerevisiae*. Genetics 174: 555-573.
8. Zhang, D.H., et al. 2006. The human PIF1 helicase, a potential *Escherichia coli* RecD homologue, inhibits telomerase activity. Nucleic Acids Res. 34: 1393-1404.

CHROMOSOMAL LOCATION

Genetic locus: Pif1 (mouse) mapping to 9 C.

PRODUCT

PIF1 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 PIF1 shRNA Plasmid (m): sc-76135-SH and PIF1 shRNA (m) Lentiviral Particles: sc-76135-V as alternate gene silencing products.

For independent verification of PIF1 (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-76135A, sc-76135B and sc-76135C.

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

PIF1 siRNA (m) is recommended for the inhibition of PIF1 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

PIF1 (F-10): sc-48377 is recommended as a control antibody for monitoring of PIF1 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 PIF1 gene expression knockdown using RT-PCR Primer: PIF1 (m)-PR: sc-76135-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.