

DNA pol ϵ A siRNA (h): sc-43728

BACKGROUND

DNA replication, recombination and repair, all of which are necessary for genome stability, require the presence of exonucleases. In DNA replication, these enzymes are involved in the processing of Okazaki fragments, whereas in DNA repair they function to excise damaged DNA fragments and correct recombinational mismatches. Exonucleases involved in these processes include DNA polymerases, including DNA pol δ and ϵ . DNA pol δ consists of two subunits: p125, which interacts directly with the sliding DNA clamp protein PCNA; and p50. DNA pol δ can be regulated by cell cycle proteins. DNA pol ϵ is a multiple subunit enzyme, the catalytic subunit of which is encoded by the POL2 gene. The exact reactions catalyzed by DNA pol δ and ϵ on leading and lagging strands have not yet been elucidated.

REFERENCES

1. Lee, M.Y., et al. 1984. Further studies on calf thymus DNA polymerase δ purified to homogeneity by a new procedure. *Biochemistry* 23: 1906-1913.
2. Hamatake, R.K., et al. 1990. Purification and characterization of DNA polymerase II from the yeast *Saccharomyces cerevisiae*. Identification of the catalytic core and a possible holoenzyme form of the enzyme. *J. Biol. Chem.* 265: 4072-4083.
3. Goulian, M., et al. 1990. Discontinuous DNA synthesis by purified mammalian proteins. *J. Biol. Chem.* 265: 18461-18471.
4. Morrison, A., et al. 1990. A third essential DNA polymerase in *S. cerevisiae*. *Cell* 62: 1143-1151.
5. Zeng, X.R., et al. 1994. Regulation of human DNA polymerase δ during the cell cycle. *J. Biol. Chem.* 269: 24027-24033.
6. Johnson, R.E., et al. 1995. Requirement of the yeast RTH1 5' to 3' exonuclease for the stability of simple repetitive DNA. *Science* 269: 238-240.
7. Zhang, P., et al. 1999. Direct interaction of proliferating cell nuclear antigen with the p125 catalytic subunit of mammalian DNA polymerase δ . *J. Biol. Chem.* 274: 26647-26653.

CHROMOSOMAL LOCATION

Genetic locus: POLE (human) mapping to 12q24.33.

PRODUCT

DNA pol ϵ A 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DNA pol ϵ A shRNA Plasmid (h): sc-43728-SH and DNA pol ϵ A shRNA (h) Lentiviral Particles: sc-43728-V as alternate gene silencing products.

For independent verification of DNA pol ϵ A (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-43728A, sc-43728B and sc-43728C.

PROTOCOLS

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

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

APPLICATIONS

DNA pol ϵ A siRNA (h) is recommended for the inhibition of DNA pol ϵ A 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 μ M in 66 μ 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

DNA pol ϵ A (D-10): sc-390785 is recommended as a control antibody for monitoring of DNA pol ϵ A 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 DNA pol ϵ A gene expression knockdown using RT-PCR Primer: DNA pol ϵ A (h)-PR: sc-43728-PR (20 μ l, 578 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Martinez-Ordoñez, A., et al. 2018. Breast cancer metastasis to liver and lung is facilitated by Pit-1-CXCL12-CXCR4 axis. *Oncogene* 37: 1430-1444.

RESEARCH USE

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