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DNA pol ϵ A shRNA (h) Lentiviral Particles: sc-43728-V

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

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- 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.
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CHROMOSOMAL LOCATION

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

PRODUCT

DNA pol ϵ A shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see DNA pol ϵ A siRNA (h): sc-43728 and DNA pol ϵ A shRNA Plasmid (h): sc-43728-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

DNA pol ϵ A shRNA (h) Lentiviral Particles is recommended for the inhibition of DNA pol ϵ A expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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