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Diagnostik & molekulare Diagnostik



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LEDGF/p75p52 shRNA (h) Lentiviral Particles: sc-44991-V



The Power to Question

BACKGROUND

PSIP1 (PC4 and SFRS1 interacting protein 1), also known as CLL-associated antigen KW-7, PSIP2, LEDGF (lens epithelium-derived growth factor), PAIP, DFS70 (dense fine speckles 70 kDa protein) or transcriptional coactivator p75/p52, is a 530 amino acid nuclear protein that associates with chromatin throughout the cell cycle. Functioning as a transcriptional coactivator that complexes with the human immunodeficiency virus type 1 (HIV-1) integrase, PSIP1 is essential for the nuclear localization and chromosomal association of viral proteins. As the primary integrase-to-chromatin tethering factor for HIV-1, PSIP1 is responsible for the cellular trafficking of lentiviral integrases. During apoptosis, PSIP1 is cleaved at three sites by caspase-3 and caspase-7, contributing to the pathogenesis of atopic disorders.

REFERENCES

- Wu, X., et al. 2002. Caspase cleavage of the nuclear autoantigen LEDGF/p75 abrogates its pro-survival function: implications for autoimmunity in atopic disorders. Cell Death Differ. 9: 915-925.
- Cherepanov, P., et al. 2003. HIV-1 integrase forms stable tetramers and associates with LEDGF/p75 protein in human cells. J. Biol. Chem. 278: 372-381
- Cherepanov, P., et al. 2004. Identification of an evolutionarily conserved domain in human lens epithelium-derived growth factor/transcriptional co-activator p75 (LEDGF/p75) that binds HIV-1 integrase. J. Biol. Chem. 279: 48883-48892
- Llano, M., et al. 2004. Lens epithelium-derived growth factor/p75 prevents proteasomal degradation of HIV-1 integrase. J. Biol. Chem. 279: 55570-55577.
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CHROMOSOMAL LOCATION

Genetic locus: PSIP1 (human) mapping to 9p22.3.

PRODUCT

LEDGF/p75p52 shRNA (h) Lentiviral Particles are concentrated, transduction-ready viral particles containing a target-specific construct that encodes a 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μl frozen stock containing 1.0 x 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 LEDGF/p75p52 siRNA (h): sc-44991 and LEDGF/p75p52 shRNA Plasmid (h): sc-44991-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

LEDGF/p75p52 shRNA (h) Lentiviral Particles is recommended for the inhibition of LEDGF expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 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

PSIP1 (C-16): sc-33371 is recommended as a control antibody for monitoring of PSIP1 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor LEDGF gene expression knockdown using RT-PCR Primer: LEDGF/p75p52 (h)-PR: sc-44991-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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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