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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

Pellino 1 shRNA (h) Lentiviral Particles: sc-44401-V

BACKGROUND

Mammalian Pellino proteins 1-3 (pellino *Drosophila* homolog 1-3) are scaffolding components within Toll-like receptor (TLR) and Interleukin-1 (IL-1) receptor signaling cascades. Pellino 1 and 3 interact with complexes that also contain IL-1R-associated kinase 4 (IRAK4) and tumor necrosis factor receptor-associated factor 6 (TRAF6). Pellino 1, 2 and 3 interact with TAK1 (TGF β activated kinase 1). Pellino 2 can initiate mitogen activated protein kinase pathways leading to activation of AP-1 and Elk-1. Pellino 3 promotes translocation of MAPK-activated protein kinase2 from the nucleus to the cytoplasm and activates transcription factor CREB in a p38 MAPK-dependent manner. Pellino 3 physically interacts with NF κ B-inducing kinase (NIK) in an IL-1-dependent manner and leads to activation of c-Jun, Elk-1 and c-Jun N-terminal kinase.

REFERENCES

- Rich, T., et al. 2000. Pellino-related sequences from *Caenorhabditis elegans* and *Homo sapiens*. *Immunogenetics* 52: 145-149.
- Resch, K., et al. 2001. Assignment of homologous genes, Peli1/PELI1 and Peli2/PELI2, for the Pelle adaptor protein Pellino to mouse chromosomes 11 and 14 and human chromosomes 2p13.3 and 14q21, respectively, by physical and radiation hybrid mapping. *Cytogenet. Cell Genet.* 92: 172-174.
- Yu, K.Y., et al. 2002. Cutting edge: mouse pellino-2 modulates IL-1 and lipopolysaccharide signaling. *J. Immunol.* 169: 4075-4078.
- Jiang, Z., et al. 2003. Pellino 1 is required for interleukin-1 (IL-1)-mediated signaling through its interaction with the IL-1 receptor-associated kinase 4 (IRAK4)-IRAK-tumor necrosis factor receptor-associated factor 6 (TRAF6) complex. *J. Biol. Chem.* 278: 10952-10956.
- Jensen, L.E., et al. 2003. Pellino3, a novel member of the Pellino protein family, promotes activation of c-Jun and Elk-1 and may act as a scaffolding protein. *J. Immunol.* 171: 1500-1506.

CHROMOSOMAL LOCATION

Genetic locus: PELI1 (human) mapping to 2p14.

PRODUCT

Pellino 1 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 Pellino 1 siRNA (h): sc-44401 and Pellino 1 shRNA Plasmid (h): sc-44401-SH as alternate gene silencing products.

APPLICATIONS

Pellino 1 shRNA (h) Lentiviral Particles is recommended for the inhibition of Pellino 1 expression in human cells.

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.

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

Pellino 1 (V-15): sc-31618 is recommended as a control antibody for monitoring of Pellino 1 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 Pellino 1 gene expression knockdown using RT-PCR Primer: Pellino 1 (h)-PR: sc-44401-PR (20 μ l). 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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