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NALP1 shRNA (h) Lentiviral Particles: sc-45479-V

BACKGROUND

NACHT-, LRR- and PYD-containing protein 1 (NALP1), also designated caspase recruitment domain protein 7, is a cytoplasmic protein. NALP1 contains a putative nucleotide binding site, a region of leucine-rich repeats, and death domain folds at both termini, providing protein/protein association functions such as caspase recruitment. NALP1 is involved in the innate immune response and is a component of the inflammasome. It forms cytoplasmic structures called death effector filaments and enhances APAF1 and cytochrome c-dependent activation of pro-caspase-9 and consecutive apoptosis. NALP1 is widely expressed in thymus, heart, spleen and peripheral blood leukocytes.

REFERENCES

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- Tschopp, J., Martinon, F. and Burns, K. 2003. NALPs: a novel protein family involved in inflammation. *Nat. Rev. Mol. Cell Biol.* 4: 95-104.
- Damiano, J.S., Oliveira, V., Welsh, K. and Reed, J.C. 2004. Heterotypic interactions among NACHT domains: implications for regulation of innate immune responses. *Biochem. J.* 381: 213-219.
- Sanz, C., Calasanz, M.J., Andreu, E., Richard, C., Prosper, F. and Fernandez-Luna, J.L. 2004. NALP1 is a transcriptional target for cAMP-response-element-binding protein (CREB) in myeloid leukaemia cells. *Biochem. J.* 384: 281-286.

CHROMOSOMAL LOCATION

Genetic locus: NALP1 (human) mapping to 17p13.2.

PRODUCT

NALP1 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 µl 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 NALP1 siRNA (h): sc-45479 and NALP1 shRNA Plasmid (h): sc-45479-SH as alternate gene silencing products.

APPLICATIONS

NALP1 shRNA (h) Lentiviral Particles is recommended for the inhibition of NALP1 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl 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

NALP1 (B-2): sc-166368 is recommended as a control antibody for monitoring of NALP1 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 NALP1 gene expression knockdown using RT-PCR Primer: NALP1 (h)-PR: sc-45479-PR (20 µl, 471 bp). 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.

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.

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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