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NOD2 shRNA (m) Lentiviral Particles: sc-44983-V

BACKGROUND

The mammalian homologs of the CED-4 proteins, Apaf-1 (CED-4), NOD1 (CARD4) and NOD2, contain a caspase recruitment domain (CARD) and a putative nucleotide binding domain, signified by a consensus Walker's A box (P-loop) and B box (Mg²⁺-binding site). NOD1 contains a putative regulatory domain and multiple leucine-rich repeats. NOD1 is a member of a growing family of intracellular proteins which share structural homology to the apoptosis regulator Apaf-1. NOD1 associates with the CARD-containing kinase RICK and activates NFκB. The self-association of NOD1 mediates proximity of RICK and the interaction of RICK with IKKγ. In addition, NOD1 binds to multiple caspases with long prodomains, but specifically activates caspase-9 and promotes caspase-9-induced apoptosis. NOD2 is composed of two N-terminal CARDS, a nucleotide-binding domain and multiple C-terminal leucine-rich repeats. The expression of NOD2 is highly restricted to monocytes, and activates NFκB in response to bacterial lipopolysaccharides.

REFERENCES

- Bertin, J., et al. 1999. Human CARD4 protein is a novel CED-4/Apaf-1 cell death family member that activates NFκB. *J. Biol. Chem.* 274: 12955-12958.
- Inohara, N., et al. 1999. NOD1, an Apaf-1-like activator of caspase-9 and nuclear factor κB. *J. Biol. Chem.* 274: 14560-14567.
- Inohara, N., et al. 2000. An induced proximity model for NFκB activation in the NOD1/RICK and RIP signaling pathways. *J. Biol. Chem.* 275: 27823-27831.
- Inohara, N., et al. 2000. Human NOD1 confers responsiveness to bacterial lipopolysaccharides. *J. Biol. Chem.* 276: 2551-2554.
- Ogura, Y., et al. 2001. NOD2, a NOD1/Apaf-1 family member that is restricted to monocytes and activates NFκB. *J. Biol. Chem.* 276: 4812-4818.
- Hlaing, T., et al. 2001. Molecular cloning and characterization of DEFCAP-L and -S, two isoforms of a novel member of the mammalian CED-4 family of apoptosis proteins. *J. Biol. Chem.* 276: 9230-9238.

CHROMOSOMAL LOCATION

Genetic locus: Card15 (mouse) mapping to 8 C3.

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.

PRODUCT

NOD2 shRNA (m) 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 x 10⁶ 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 NOD2 siRNA (m): sc-44983 and NOD2 shRNA Plasmid (m): sc-44983-SH as alternate gene silencing products.

APPLICATIONS

NOD2 shRNA (m) Lentiviral Particles is recommended for the inhibition of NOD2 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μl frozen viral stock containing 1.0 x 10⁶ 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

NOD2 (H-300): sc-30199 is recommended as a control antibody for monitoring of NOD2 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NOD2 gene expression knockdown using RT-PCR Primer: NOD2 (m)-PR: sc-44983-PR (20 μl, 467 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.

PROTOCOLS

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