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NOD2 siRNA (h): sc-43973

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 lipopoly-saccharides.

CHROMOSOMAL LOCATION

Genetic locus: NOD2 (human) mapping to 16q12.1.

PRODUCT

NOD2 siRNA (h) is a target-specific 19-25 nt siRNA designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NOD2 shRNA Plasmid (h): sc-43973-SH and NOD2 shRNA (h) Lentiviral Particles: sc-43973-V as alternate gene silencing products.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μl of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μl of RNase-free water makes a 10 μM solution in a 10 μM Tris-HCL, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NOD2 siRNA (h) is recommended for the inhibition of NOD2 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μM in 66 μl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

NOD2 (2D9): sc-56168 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 reagents are recommended: 1) Western Blotting: use m-IgGκc BP-HRP: sc-516102 or m-IgGκc BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκc BP-FITC: sc-516140 or m-IgGκc BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NOD2 gene expression knockdown using RT-PCR Primer: NOD2 (h)-PR: sc-43973-PR (20 μl, 446 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Chiu, Y.C., et al. 2009. Peptidoglycan enhances IL-6 production in human synovial fibroblasts via TLR2 receptor, focal adhesion kinase, Akt, and AP-1-dependent pathway. *J. Immunol.* 183: 2785-2792.
- Leung, C.H., et al. 2009. Butyrate mediates nucleotide-binding and oligomerisation domain (NOD) 2-dependent mucosal immune responses against peptidoglycan. *Eur. J. Immunol.* 39: 3529-3537.
- Watanabe, T., et al. 2012. Involvement of activation of Toll-like receptors and nucleotide-binding oligomerization domain-like receptors in enhanced IgG₄ responses in autoimmune pancreatitis. *Arthritis Rheum.* 64: 914-924.
- Venza, I., et al. 2013. NOD2 triggers PGE2 synthesis leading to IL-8 activation in *Staphylococcus aureus*-infected human conjunctival epithelial cells. *Biochem. Biophys. Res. Commun.* 440: 551-557.
- Tan, G., et al. 2016. NOD2 up-regulates TLR2-mediated IL-23p19 expression via NFκB subunit c-Rel in Paneth cell-like cells. *Oncotarget* 7: 63651-63660.
- Saxena, A., et al. 2017. Absence of the NOD2 protein renders epithelia more susceptible to barrier dysfunction due to mitochondrial dysfunction. *Am. J. Physiol. Gastrointest. Liver Physiol.* 313: G26-G38.
- Cañas, M.A., et al. 2018. Outer membrane vesicles from probiotic and commensal *Escherichia coli* activate NOD1-mediated immune responses in intestinal epithelial cells. *Front. Microbiol.* 9: 498.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

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