SANTA CRUZ BIOTECHNOLOGY, INC.

NOD1 siRNA (m2): sc-270279

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 (Mg^{2+}-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.

REFERENCES

1. 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.
2. Inohara, N., et al. 1999. NOD1, an Apaf-1-like activator of caspase-9 and nuclear factor κB. J. Biol. Chem. 274: 14560-14567.
3. 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.
4. Inohara, N., et al. 2000. Human NOD1 confers responsiveness to bacterial lipopolysaccharides. J. Biol. Chem. 276: 2551-2554.
5. 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.
6. 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: Nod1 (mouse) mapping to 6 B3.

PRODUCT

NOD1 siRNA (m2) is a pool of 3 target-specific 19-25 nt siRNAs 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 NOD1 shRNA Plasmid (m2): sc-270279-SH and NOD1 shRNA (m2) Lentiviral Particles: sc-270279-V as alternate gene silencing products.

For independent verification of NOD1 (m2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270279A, sc-270279B and sc-270279C.

PROTOCOLS

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

NOD1 siRNA (m2) is recommended for the inhibition of NOD1 expression in mouse 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

NOD1 (B-4): sc-398696 is recommended as a control antibody for monitoring of 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 NOD1 gene expression knockdown using RT-PCR Primer: NOD1 (m2)-PR: sc-270279-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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