



# Apaf-1 siRNA (h): sc-29201

## 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<sup>2+</sup>-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, Nod-1 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

- 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. 2000. Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NFκB. *J. Biol. Chem.* 276: 4812-4818.

## CHROMOSOMAL LOCATION

Genetic locus: APAF1 (human) mapping to 12q23.1.

## PRODUCT

Apaf-1 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 Apaf-1 shRNA Plasmid (h): sc-29201-SH and Apaf-1 shRNA (h) Lentiviral Particles: sc-29201-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

Apaf-1 siRNA (h) is recommended for the inhibition of Apaf-1 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

Apaf-1 (5E11): sc-65891 is recommended as a control antibody for monitoring of Apaf-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).

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Apaf-1 gene expression knockdown using RT-PCR Primer: Apaf-1 (h)-PR: sc-29201-PR (20 μl, 530 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

- Meyer, K., et al. 2005. Inhibition of hepatitis C virus core protein expression in immortalized human hepatocytes induces cytochrome c-independent increase in Apaf-1 and caspase-9 activation for cell death. *Virology* 336: 198-207.
- Filomenko, R., et al. 2006. Caspase-10 involvement in cytotoxic drug-induced apoptosis of tumor cells. *Oncogene* 25: 7635-7645.
- Wang, J., et al. 2012. Simultaneous modulation of COX-2, p300, Akt, and Apaf-1 signaling by melatonin to inhibit proliferation and induce apoptosis in breast cancer cells. *J. Pineal Res.* 53: 77-90.
- Tang, Y., et al. 2015. The role of miR-19b in the inhibition of endothelial cell apoptosis and its relationship with coronary artery disease. *Sci. Rep.* 5: 15132.
- Sun, X., et al. 2015. MicroRNA-221 accelerates the proliferation of laryngeal cancer cell line Hep-2 by suppressing Apaf-1. *Oncol. Rep.* 33: 1221-1226.

## RESEARCH USE

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