

Apaf-1 siRNA (m): sc-37147

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, 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 lipopolysaccharides.

REFERENCES

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- Inohara, N., et al. 2000. Human NOD1 confers responsiveness to bacterial lipopolysaccharides. *J. Biol. Chem.* 276: 2551-2554.
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CHROMOSOMAL LOCATION

Genetic locus: Apaf1 (mouse) mapping to 10 C2.

PRODUCT

Apaf-1 siRNA (m) 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 Apaf-1 shRNA Plasmid (m): sc-37147-SH and Apaf-1 shRNA (m) Lentiviral Particles: sc-37147-V as alternate gene silencing products.

For independent verification of Apaf-1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-37147A, sc-37147B and sc-37147C.

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).

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 Apaf-1 gene expression knockdown using RT-PCR Primer: Apaf-1 (m)-PR: sc-37147-PR (20 μl, 530 bp). 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.

PROTOCOLS

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