

#6229 Store at -20°C

SignalSilence® Apaf-1 siRNA I



✓ 10 µM in 300 µl (100 transfections)

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For Research Use Only. Not For Use In Diagnostic Procedures.

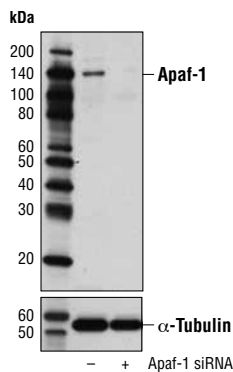
Species Cross-Reactivity: H

Description: SignalSilence® Apaf-1 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit Apaf-1 expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

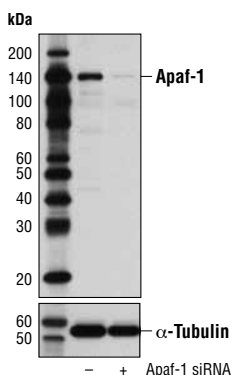
Background: Apoptotic protease activating factor 1 (Apaf-1), originally identified as the mammalian homolog of the *C. elegans* apoptotic regulatory protein CED-4, is an important signaling protein involved in the activation of caspase-9 during apoptosis (1). Cytosolic Apaf-1 forms a complex with caspase-9 in the presence of cytochrome c and dATP, ultimately leading to caspase-9 activation and subsequent activation of caspase-3 (2,3). The protein contains an amino-terminal CARD domain, a central CED-4 homology domain, and multiple WD-40 repeats at the carboxy-terminus. Several isoforms of Apaf-1 are expressed through alternative splicing generating a small insert following the CARD domain as well as an extra WD-40 repeat (4). The critical role of Apaf-1 has been observed in knock-out mice which show widespread defects in apoptosis and resistance to a variety of apoptotic stimuli (5,6).

Directions for Use: CST recommends transfection with 100 nM Apaf-1 siRNA I 48 to 72 hours prior to cell lysis. For transfection procedure, follow protocol provided by the transfection reagent manufacturer. Please feel free to contact CST with any questions on use.

Quality Control: Oligonucleotide synthesis is monitored base by base through trityl analysis to ensure appropriate coupling efficiency. The oligo is subsequently purified by affinity-solid phase extraction. The annealed RNA duplex is further analyzed by mass spectrometry to verify the exact composition of the duplex. Each lot is compared to the previous lot by mass spectrometry to ensure maximum lot-to-lot consistency.



Western blot analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or SignalSilence® Apaf-1 siRNA I (+), using Apaf-1 (D5C3) Rabbit mAb #8969 (upper) or α -Tubulin (11H10) Rabbit mAb #2125 (lower). The Apaf-1 (D5C3) Rabbit mAb confirms silencing of Apaf-1 expression, while the α -Tubulin (11H10) Rabbit mAb is used as a loading control.



Western blot analysis of extracts from HeLa cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or SignalSilence® Apaf-1 siRNA I (+), using Apaf-1 (D7G4) Rabbit mAb #8723 (upper) or α -Tubulin (11H10) Rabbit mAb #2125 (lower). The Apaf-1 (D7G4) Rabbit mAb confirms silencing of Apaf-1 expression, while the α -Tubulin (11H10) Rabbit mAb is used as a loading control.

Entrez-Gene ID #317
Swiss-Prot Acc. #O14727

Storage: Apaf-1 siRNA I is supplied in RNase-free water. Aliquot and store at -20°C.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

- (1) Zou, H. et al. (1997) *Cell* 90, 405-13.
- (2) Zou, H. et al. (1999) *J Biol Chem* 274, 11549-56.
- (3) Saleh, A. et al. (1999) *J Biol Chem* 274, 17941-5.
- (4) Benedict, M.A. et al. (2000) *J Biol Chem* 275, 8461-8.
- (5) Ceconi, F. et al. (1998) *Cell* 94, 727-37.
- (6) Yoshida, H. et al. (1998) *Cell* 94, 739-50.

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Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.