

SignalSilence® APC3 siRNA I

✓ 10 µM in 300 µl
 (3 nmol)



Orders ■ 877-616-CELL (2355)
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New 08/12

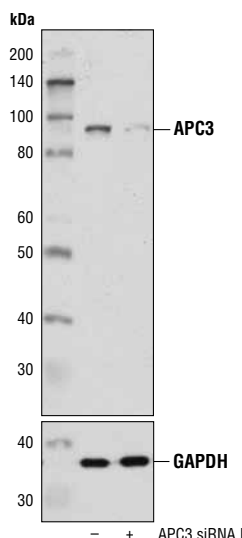
For Research Use Only. Not For Use In Diagnostic Procedures.

Species Cross-Reactivity: H, (Mk)

Description: SignalSilence® APC3 siRNA I from Cell Signaling Technology (CST) allows the researcher to specifically inhibit APC3 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: Cell proliferation in all eukaryotic cells depends strictly upon the ubiquitin ligase (E3) activity of the anaphase promoting complex/cyclosome (APC/C), whose main function is to trigger the transition of the cell cycle from metaphase to anaphase. APC/C is a 1.5 MDa protein complex found in the nucleus of interphase cells. This complex diffuses throughout the cytoplasm and associates with parts of the spindle apparatus during mitosis. APC/C performs its various functions by promoting the assembly of polyubiquitin chains on substrate proteins, which targets these proteins for degradation by the 26S proteasome (1,2). In humans, twelve different APC/C subunits have been identified. Like all E3 enzymes, APC/C utilizes ubiquitin residues that have been activated by E1 enzymes and then transferred to E2 enzymes. Indeed APC/C has been shown to transiently interact with UBCH5 and UBCH10 E2 enzymes, in part, via the RING-finger domain-containing subunit, APC11 (3-5). In addition to E2 enzymes, APC/C activity is also strictly dependent upon one of several cofactors that associate with APC/C during specific phases of the cell cycle. The best studied of these are Cdc20 and Cdh1/FZR1, which contain a C-terminal WD40 domain and participate in the recognition of APC/C substrates by interacting with specific recognition elements in these substrates (6), called D-boxes (7) and KEN-boxes (8).

Anaphase-promoting complex subunit 3 (APC3) is the human homolog of *Saccharomyces cerevisiae* CDC27 (9), and like APC8/CDC23 and APC6/CDC16, APC3 is a component of the tetratricopeptide (TPR) subcomplex of the APC/C. It has been demonstrated that the binding of Cdh1/FZR1 to the APC/C depends upon the presence of APC3, implying that APC/C is activated by the association of Cdh1/FZR1 with APC3, which enables APC/C to recognize the D-box of substrates (10,11). APC3 has been shown to be localized to the centrosome at all stages of the mammalian cell cycle, and to the mitotic spindle, suggesting that APC3 plays a critical role for the transition from metaphase to anaphase during mitosis (12). During mitosis, APC3 becomes phosphorylated at numerous sites. This is predicted to change the surface charge distribution significantly such that these modifications could either induce structural changes within the APC/C by altering subunit-subunit interactions or they could change the



Western blot analysis of extracts from 293T cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-) or SignalSilence® APC3 siRNA I (+), using APC3 Antibody #9063 (upper) or GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). The APC3 Antibody confirms silencing of APC3 expression, while the GAPDH (D16H11) XP® Rabbit mAb is used as a loading control.

affinity for molecules that only transiently associate with the APC/C, such as Cdh1/FZR1 (13,14).

Specificity/Sensitivity: SignalSilence® APC3 siRNA I inhibits human and monkey APC3 expression.

Directions for Use: CST recommends transfection with 100 nM SignalSilence® APC3 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.

Each vial contains the equivalent of 100 transfections, which corresponds to a final siRNA concentration of 100 nM per transfection in a 24-well plate with a total volume of 300 µl per well.

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.

Entrez-Gene ID #996
 Swiss-Prot Acc. #P30260

Storage: APC3 siRNA I is supplied in RNase-free water. Aliquot and store at -20°C.

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

Background References:

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- (3) Carroll, C.W. and Morgan, D.O. (2002) *Nat Cell Biol* 4, 880-7.
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- (5) Leversen, J.D. et al. (2000) *Mol Biol Cell* 11, 2315-25.
- (6) Kraft, C. et al. (2005) *Mol Cell* 18, 543-53.
- (7) Glotzer, M. et al. (1991) *Nature* 349, 132-8.
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- (10) Kraft, C. et al. (2005) *Mol Cell* 18, 543-53.
- (11) Vodermaier, H.C. et al. (2003) *Curr Biol* 13, 1459-68.
- (12) Tugendreich, S. et al. (1995) *Cell* 81, 261-8.
- (13) Topper, L.M. et al. *Cell Cycle* 1, 282-92.
- (14) Kraft, C. et al. (2003) *EMBO J* 22, 6598-609.

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.