#12530 Store at -20°C

APC3 (D3I1V) Rabbit mAb

☑ 100 µl
(10 western blots)

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New 02/13

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Entrez-Gene ID #976
Swiss-Prot Acc. #P30260

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP Endogenous	H, M, R, Mk, (Hm, B, Dg, Pg, Hr)	97 kDa	Rabbit IgG**

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibody.*

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:100

For product specific protocols please see the web page for this product at www.cellsignal.com.

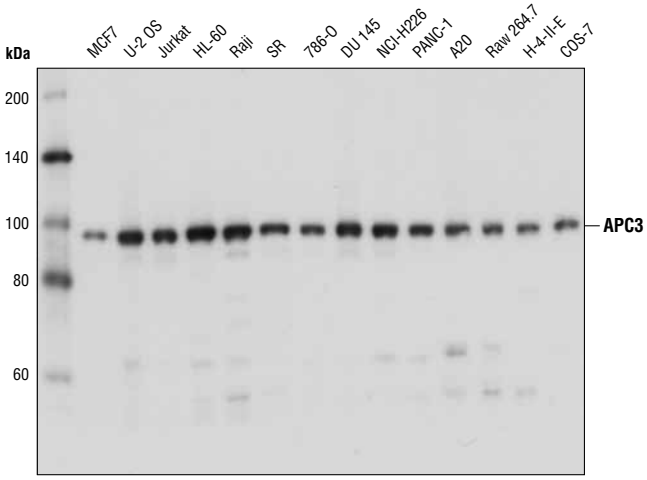
Please visit www.cellsignal.com for a complete listing of recommended complementary products.

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

Specificity/Sensitivity: APC3 (D3I1V) Rabbit mAb recognizes endogenous levels of total APC3 protein. This antibody does not cross-react with either APC8/CDC23 or APC6/CDC16.

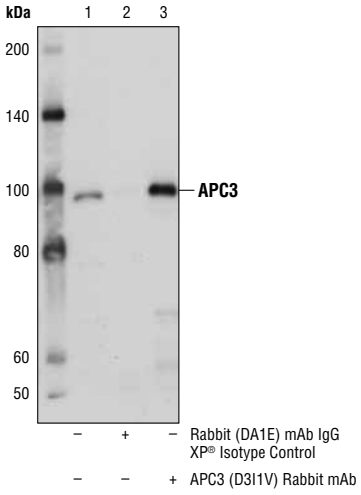
Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the carboxy terminus of human APC3 protein.



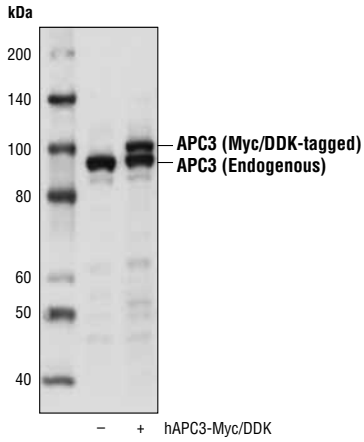
Western blot analysis of extracts from various cell lines using APC3 (D3I1V) Rabbit mAb.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

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.



Immunoprecipitation of APC3 from MCF7 cell extracts, using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or APC3 (D3I1V) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using APC3 (D3I1V) Rabbit mAb.



Western blot analysis of extracts from 293T cells, mock transfected (-) or transfected with a construct expressing Myc/DDK-tagged full-length human APC3 (hAPC3-Myc/DDK; +), using APC3 (D3I1V) Rabbit mAb.

Background References:

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(14) Kraft, C. et al. (2003) *EMBO J* 22, 6598-609.