

Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb

- Small 100 µl (10 western blots)
- Large 300 µl (30 western blots)

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IHC-F, IF-IC, F Endogenous	H, M, R, Mk, (Dg)	17, 19 kDa	Rabbit IgG**

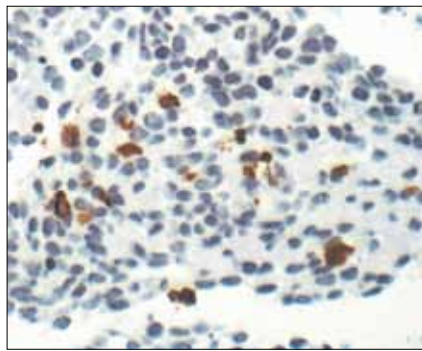
Background: Caspase-3 (CPP-32, Apoptain, Yama, SCA-1) is one of the key executioners of apoptosis, as it is either partially or totally responsible for the proteolytic cleavage of many key proteins such as the nuclear enzyme poly (ADP-ribose) polymerase (PARP) (1). Activation of caspase-3 requires proteolytic processing of its inactive zymogen into activated p17 and p12 fragments. Cleavage of caspase-3 requires aspartic acid at the P1 position (2).

Specificity/Sensitivity: Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb detects endogenous levels of the large fragment (17/19 kDa) of activated caspase-3 resulting from cleavage adjacent to Asp175. This antibody does not recognize full length caspase-3 or other cleaved caspases.

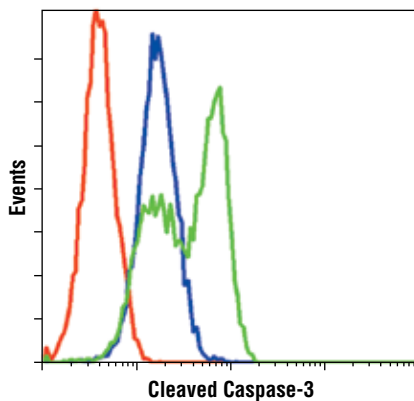
Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to amino-terminal residues adjacent to Asp175 of human caspase-3.

Background References:

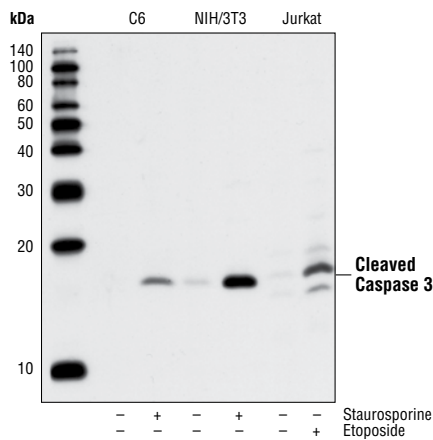
- (1) Fernandes-Alnemri, T. et al. (1994) *J. Biol. Chem.* 269, 30761–30764.
- (2) Nicholson, D.W. et al. (1995) *Nature* 376, 37–43.



Immunohistochemical staining of paraffin-embedded mouse embryo, showing cytoplasmic localization in apoptotic cells, using Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb.



Flow cytometric analysis of Jurkat cells, untreated (blue) or etoposide-treated (green), using Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb compared to a nonspecific negative control antibody (red).



◀ Western blot analysis of extracts from C6 (rat), NIH/3T3 (mouse), and Jurkat (human) cells, untreated or treated with staurosporine (1µM, 3hrs) or etoposide (25µM, 5hrs) as indicated, using Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb.

Entrez-Gene ID #836
Swiss-Prot Acc. #P42574

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.

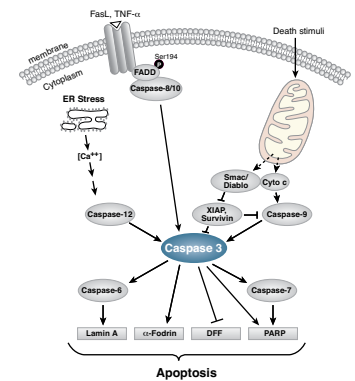
**Anti-rabbit secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:50
Immunohistochemistry (Paraffin)	1:800†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.	
Immunohistochemistry (Frozen)	1:400
Fixative:	3% Formaldehyde
Immunofluorescence (IF-IC)	1:400
Flow Cytometry	1:400

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

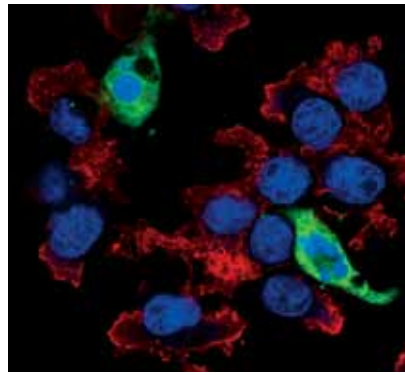
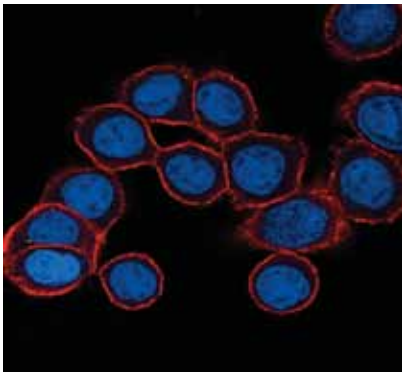
Please visit www.cellsignal.com for a complete listing of recommended companion products.



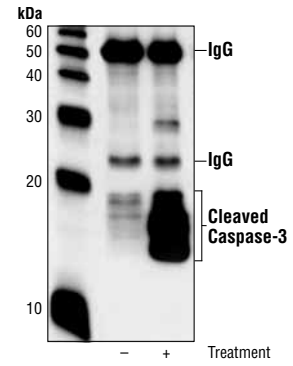
Rabbit monoclonal antibody is produced under license (granting certain rights including those under U. S. Patent No. 5,675,063) from Eptomics, Inc.

DRAQ5® is a registered trademark of Biostatus Limited.

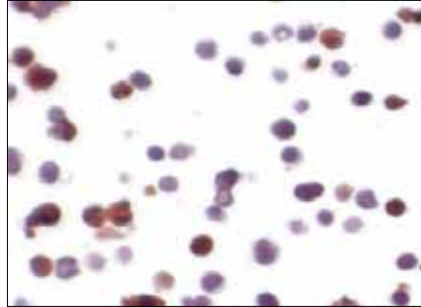
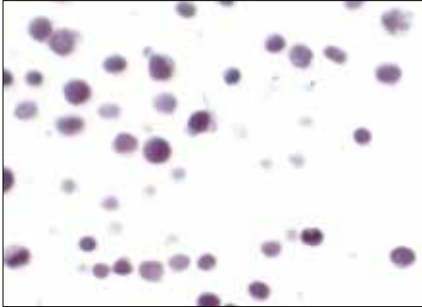
IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.



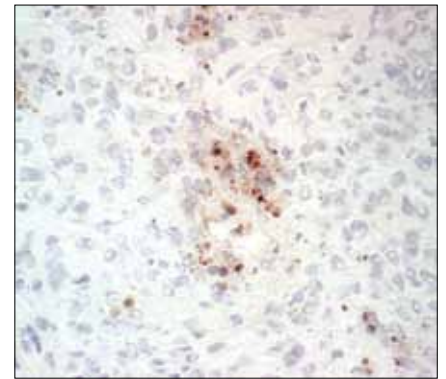
Confocal immunofluorescent images of HT-29 cells, untreated (left) or Staurosporine #9953 treated (right) labeled with Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb (green). Actin filaments have been labeled with Alexa Fluor® 555 phalloidin (red). Blue pseudocolor = DRAQ5® #4084 (fluorescent DNA dye).



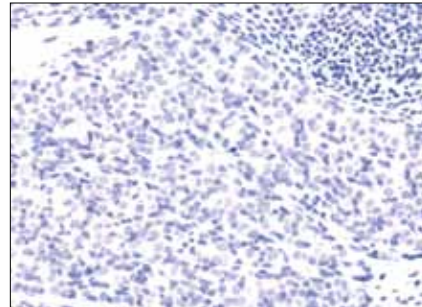
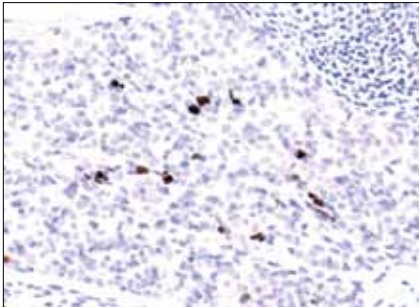
Immunoprecipitation of extracts from Jurkat cells, untreated or etoposide-treated (25uM, 5hrs), using Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb. Western blot was performed using the same antibody.



Immunohistochemical analysis using Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb on SignalSlide™ Cleaved Caspase-3 IHC Controls #8104 (paraffin-embedded Jurkat cell, untreated (left) or etoposide-treated (right)).



Immunohistochemical analysis of frozen H1650 xenograft, using Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded mouse embryo, using Cleaved Caspase-3 (Asp175) (5A1E) Rabbit mAb in the presence of control peptide (left) or Cleaved Caspase-3 (Asp175) Blocking Peptide (#1050) (right).