#9661 Store at –20°C

# Cleaved Caspase-3 (Asp175) Antibody

Cell Signaling TECHNOLOGY®

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

**Entrez-Gene ID** #836
**UniProt ID** #P42574

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

**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).

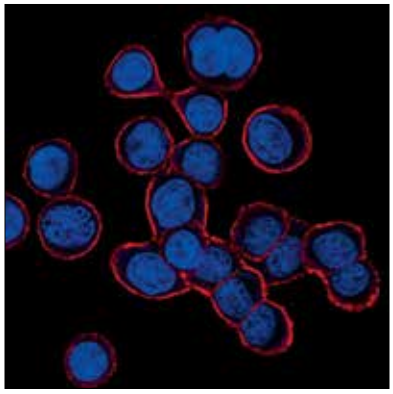
**Specificity/Sensitivity:** Cleaved Caspase-3 (Asp175) Antibody detects endogenous levels of the large fragment (17/19 kDa) of activated caspase-3 resulting from cleavage adjacent to Asp175. This antibody does not recognize full length caspase-3 or other cleaved caspases. This antibody detects non-specific caspase substrates by western blot. Non-specific labeling may be observed by immunofluorescence in specific sub-types of healthy cells in fixed-frozen tissues (e.g. pancreatic alpha-cells). Nuclear background may be observed in rat and monkey samples.

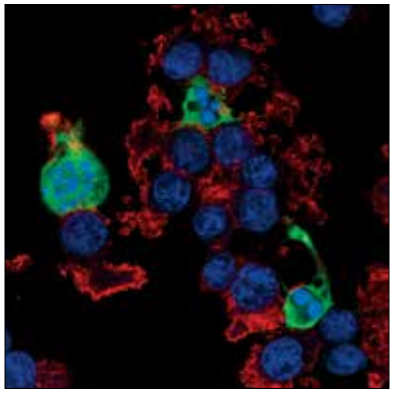
**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to amino-terminal residues adjacent to (Asp175) in human caspase-3.

**Background References:**

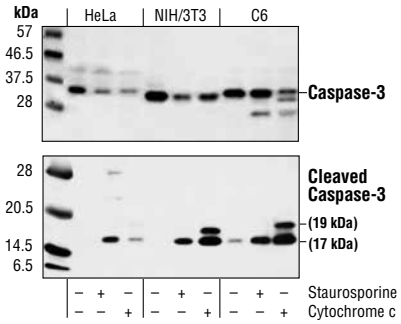
(1) Fernandes-Alnemri, T. et al. (1994) *J. Biol. Chem.* 269, 30761–30764.

(2) Nicholson, D. W. et al. (1995) *Nature* 376, 37–43.





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




◀ *Western blot analysis of extracts from HeLa, NIH/3T3 and C6 cells untreated, staurosporine-treated (1 µM in vivo) or cytochrome c-treated (0.25 mg/ml in vitro), using Caspase-3 Antibody #9662 (upper) or Cleaved Caspase-3 (Asp175) Antibody (lower).*

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. 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 |
| Immunohistochemistry (Paraffin) | 1:200† |
| 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 | 1:800 |

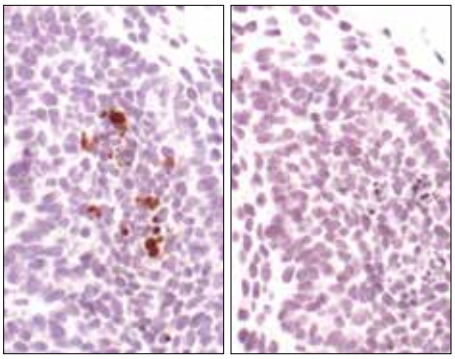
**For application specific protocols please see the web page for this product at www.cellsignal.com.**

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

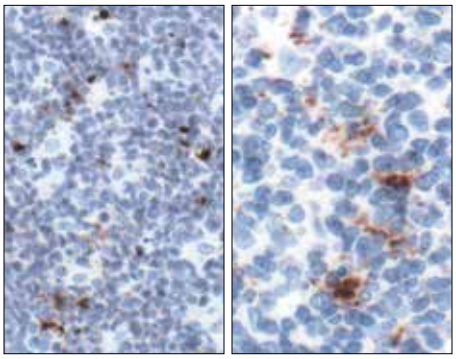
**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween®20 at 4°C with gentle shaking, overnight.**

**Applications Key:** **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

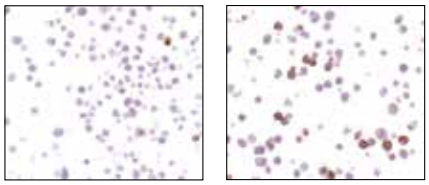
**Species Cross-Reactivity Key:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



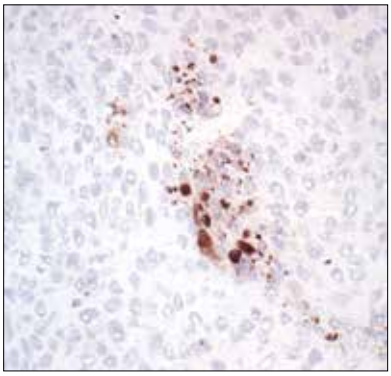
*Immunohistochemical analysis of paraffin-embedded mouse embryo, using Cleaved Caspase-3 (Asp175) Antibody preincubated with control peptide (left) or Cleaved Caspase-3 (Asp175) Blocking Peptide #1050 (right).*



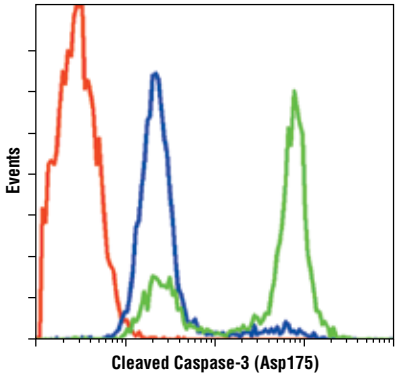
*Immunohistochemical analysis of paraffin-embedded human tonsil, showing cytoplasmic and perinuclear localization in apoptotic cells (low and high magnification), using Cleaved Caspase-3 (Asp175) Antibody.*



*Immunohistochemical analysis using Cleaved caspase-3 (Asp175) antibody on SignalSlide™ Cleaved Caspase-3 IHC controls #8104 (paraffin-embedded Jurkat cells, untreated (left) or etoposide-treated (right)).*



*Immunohistochemical analysis of frozen H1650 xenograft, using Cleaved Caspase-3 (Asp175) Antibody.*




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