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-20°C

Cleaved-PARP (Asp214) (E2T4K) Mouse mAb

#32563



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Entrez-Gene ID #142
UniProt ID #P09874

New 02/18

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Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IP, IHC-P, IF-IC, F Endogenous	H	89 kDa	Mouse IgG1**

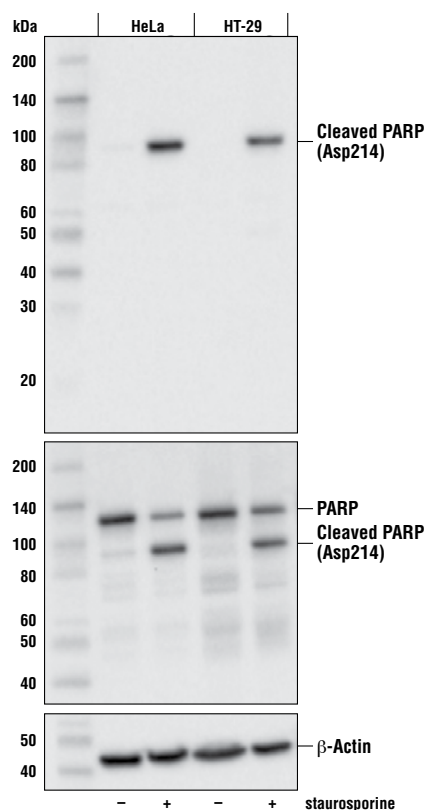
Background: PARP, a 116 kDa nuclear poly (ADP-ribose) polymerase, appears to be involved in DNA repair in response to environmental stress (1). This protein can be cleaved by many ICE-like caspases *in vitro* (2,3) and is one of the main cleavage targets of caspase-3 *in vivo* (4,5). In human PARP, the cleavage occurs between Asp214 and Gly215, which separates the PARP amino-terminal DNA binding domain (24 kDa) from the carboxy-terminal catalytic domain (89 kDa) (2,4). PARP helps cells to maintain their viability; cleavage of PARP facilitates cellular disassembly and serves as a marker of cells undergoing apoptosis (6).

Background References:

- (1) Satoh, M.S. and Lindahl, T. (1992) *Nature* 356, 356-358.
- (2) Lazebnik, Y. A. et al. (1994) *Nature* 371, 346-347.
- (3) Cohen, G.M. (1997) *Biochem. J.* 326, 1-16.
- (4) Nicholson, D. W. et al. (1995) *Nature* 376, 37-43.
- (5) Tewari, M. et al. (1995) *Cell* 81, 801-809.
- (6) Oliver, F.J. et al. (1998) *J. Biol. Chem.* 273, 33533-33539.

Specificity/Sensitivity: Cleaved PARP (Asp214) (E2T4K) Mouse mAb recognizes endogenous levels of the large fragment (89 kDa) of human PARP1 protein produced by caspase cleavage. This antibody does not recognize full-length PARP1 or other PARP isoforms.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Asp214 of human PARP protein.



Western blot analysis of extracts from serum starved HeLa or HT-29 cells, untreated (-) or treated with Staurosporine #9953 (1 μ M; +) using Cleaved PARP (Asp214) (E2T4K) Mouse mAb (upper), total PARP (46D11) Rabbit mAb #9532 (middle), or β -Actin (D6A8) Rabbit mAb #8457 (lower).

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/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-mouse secondary antibodies must be used to detect this antibody.

Recommended Antibody Dilutions:

Western blotting 1:1000

Immunoprecipitation 1:100

Immunohistochemistry (Paraffin) 1:50

Optimal IHC dilutions determined using SignalStain®

Boost IHC Detection Reagent.

Unmasking buffer: SignalStain® Citrate Unmasking Solution (10X) #14746

Antibody diluent: SignalStain® Antibody Diluent #8112

Detection reagent: SignalStain® Boost (HRP, Mouse) #8125

Immunofluorescence (IF-IC) 1:400

Fixative: 4% Formaldehyde

Permeabilization: 0.3% Triton X-100

Flow Cytometry 1:200

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

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Tween is a registered trademark of ICI Americas, Inc.

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

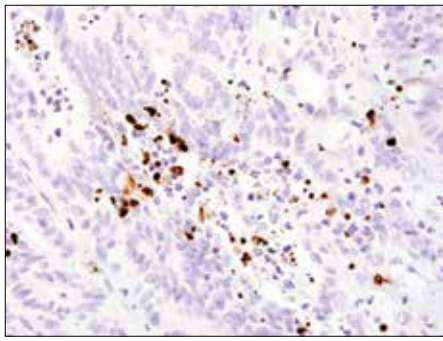
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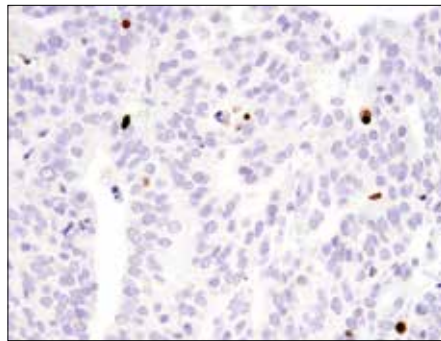
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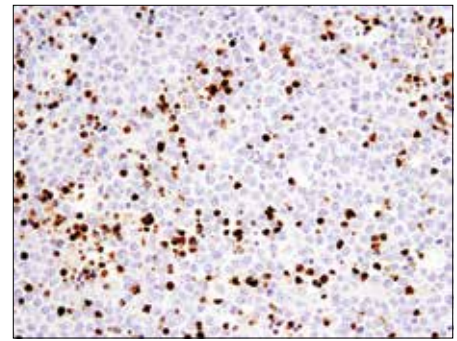
Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine 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.



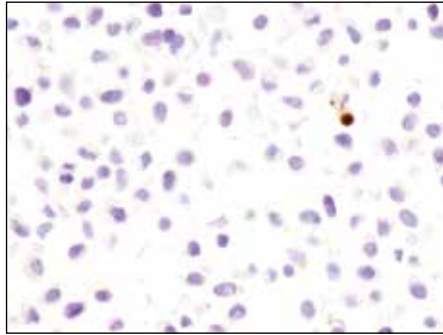
Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Cleaved-PARP (Asp214) (E2T4K) Mouse mAb.



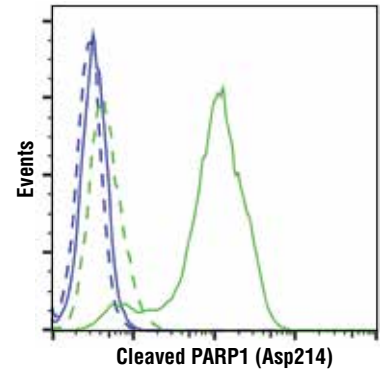
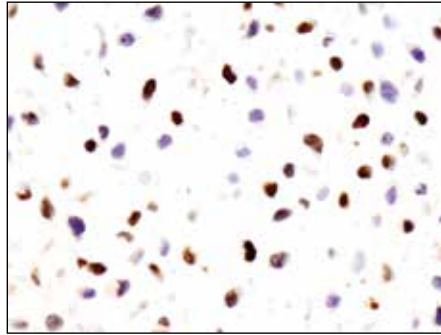
Immunohistochemical analysis of paraffin-embedded human endometrioid adenocarcinoma using Cleaved-PARP (Asp214) (E2T4K) Mouse mAb.



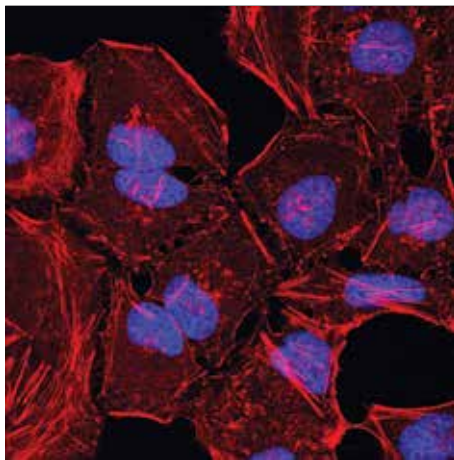
Immunohistochemical analysis of paraffin-embedded human non-Hodgkin's lymphoma using Cleaved-PARP (Asp214) (E2T4K) Mouse mAb.



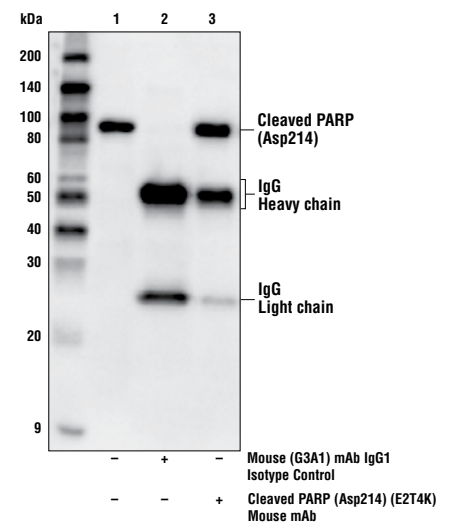
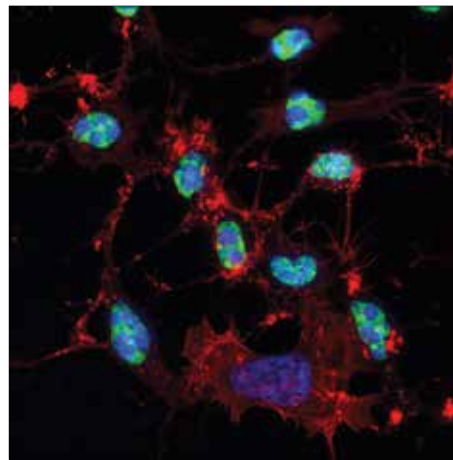
Immunohistochemical analysis of paraffin-embedded HeLa cell pellet, untreated (left) or treated with Staurosporine #9953 (right), using Cleaved-PARP (Asp214) (E2T4K) Mouse mAb.



Flow cytometric analysis of Jurkat cells, untreated (blue) or treated with etoposide (25 μ M, 18 hr; green), using Cleaved PARP1 (Asp214) (E2T4K) Mouse mAb (solid lines) or concentration-matched Mouse (G3A1) mAb IgG1 Isotype Control #5415. Anti-mouse IgG (H+L), F(ab)₂ Fragment (Alexa Fluor[®] 488 Conjugate) #4408 was used as a secondary antibody.



Confocal immunofluorescent analysis of serum-starved HeLa cells, untreated (left) or treated with Staurosporine #9953 (right), using Cleaved PARP (Asp214) (E2T4K) Mouse mAb (green). Actin filaments were labeled with DyLight[™] 554 Phalloidin #13054 (red). Blue pseudocolor = DRAQ5[®] #4084 (fluorescent DNA dye).



Immunoprecipitation of cleaved PARP (Asp214) from serum-starved HeLa cells treated with Staurosporine #9953 (1 μ M, 3 hr). Lane 1 is 10% input, lane 2 is Mouse (G3A1) mAb IgG1 Isotype Control #5415, and lane 3 is Cleaved PARP (Asp214) (E2T4K) Mouse mAb. Western blot was performed using Cleaved PARP (Asp214) (E2T4K) Mouse mAb. Anti-mouse IgG, HRP-linked Antibody #7076 was used as a secondary antibody.

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