Store at -20°C

#32563

Cleaved-PARP (Asp214) (E2T4K) Mouse mAb

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Entrez-Gene ID #142
UniProt ID #P09874

New 02/18

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

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.

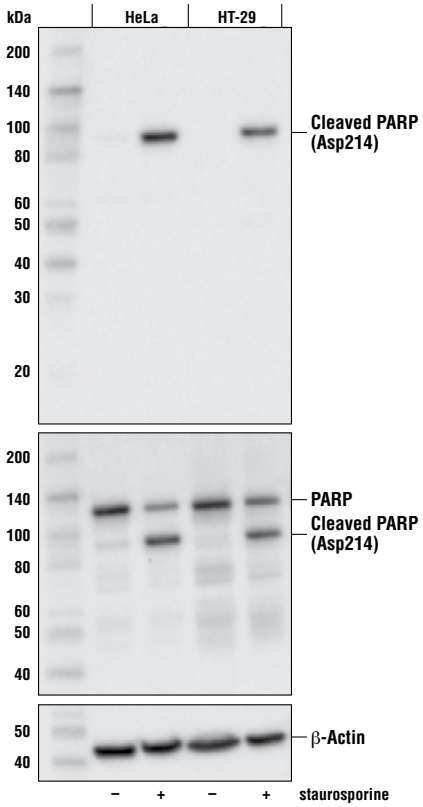
(4) Nicholson, D. W. et al. (1995) *Nature* 376, 37-43.

(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 µM ; +) 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 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-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.

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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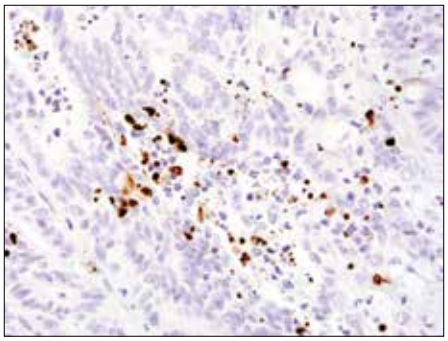
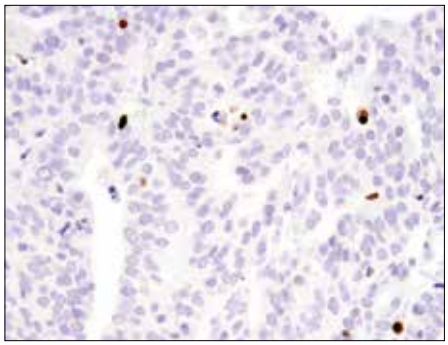
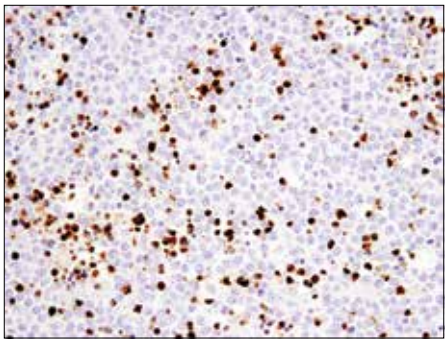
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **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.



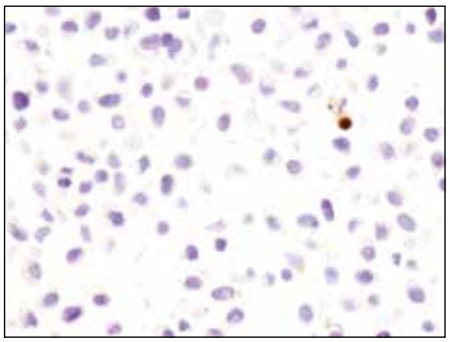
Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Cleaved-PARP (Asp214) (E2T4K) Mouse mAb.

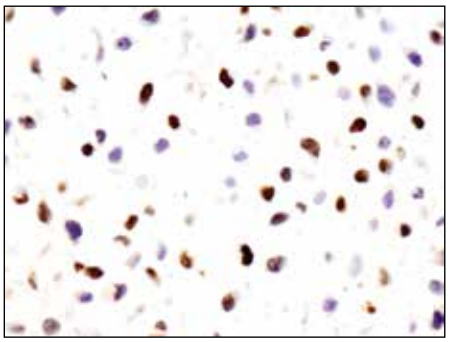


Immunohistochemical analysis of paraffin-embedded human endometrioid adenocarcinoma using Cleaved-PARP (Asp214) (E2T4K) Mouse mAb.

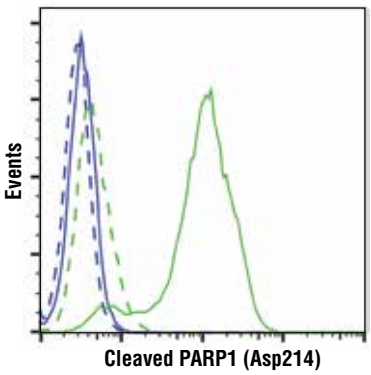


Immunohistochemical analysis of paraffin-embedded human non-Hodgkin's lymphoma using Cleaved-PARP (Asp214) (E2T4K) Mouse mAb.

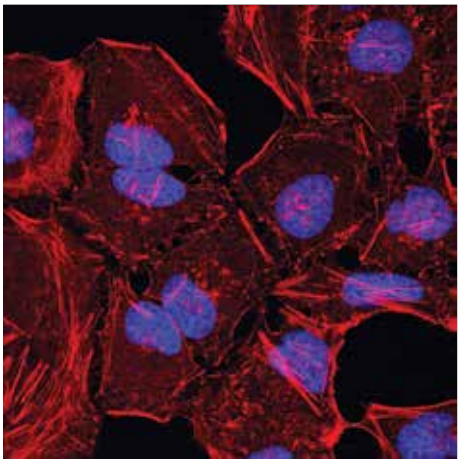


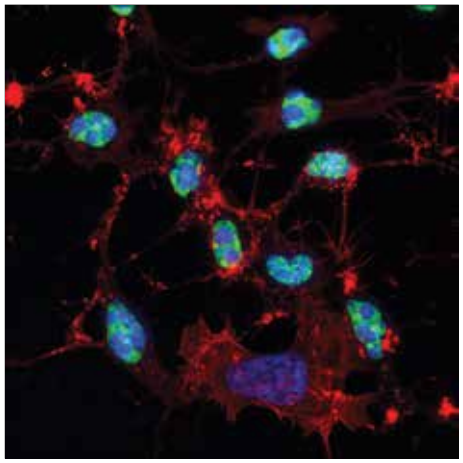


Immunohistochemical analysis of paraffin-embedded HeLa cell pellet, untreated (left) or treated with Staurosporine #9953 (right), using Cleaved-PARP (Asp214) (E2T4K) Mouse mAb.

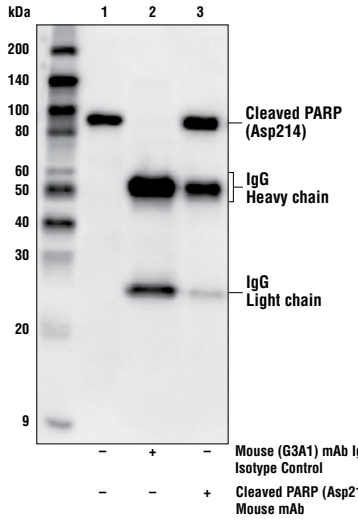


Flow cytometric analysis of Jurkat cells, untreated (blue) or treated with etoposide (25 µM, 18 hr; green), using Cleaved PARP1 (Asp214) (E2T4K) Mouse mAb (solid lines) or concentration-matched Mouse (G3A1) mAb IgG1 Isotype Control #5415. Anti-mouse IgG (H+L), F(ab')$_2$ Fragment (Alexa Fluor® 488 Conjugate) #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 µM, 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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