1/1/24, 8:14 AM Revision 7

Histone H2A.X (D17A3) XP <sup>®</sup> Rabbit mAb				3 Trask L	Orders: Support: Web:	Cell Signaling         TECHNOLOGY*         Orders:       877-616-CELL (2355)         orders@cellsignal.com         Support:       877-678-TECH (8324)         Web:       info@cellsignal.com         cellsignal.com         ne       Danvers	
For Research Use Only	Not for Use in	Diagnostic Proc	edures.				
Applications: WB, W-S, IHC-P, IF-IC, FC-FP	Reactivity: H M R Mk	Sensitivity: Endogenous	<b>MW (kDa):</b> 15	Source/Isotype: Rabbit IgG	UniProt ID: #P16104	Entrez-Gene Id: 3014	
Product Usage Information	Арј	plication		Dilution			
	We	Western Blotting				1:1000	
	Sim	Simple Western™				1:10 - 1:50	
	Imn	Immunohistochemistry (Paraffin)			:	1:50 - 1:200	
	Imn	Immunofluorescence (Immunocytochemistry)			:	1:50 - 1:200	
	Flov	w Cytometry (Fixed	l/Permeabilized)		:	1:50	
Storage	<b>rage</b> Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μg/ml BSA, 50% glycerol and less 0.02% sodium azide. Store at –20°C. Do not aliquot the antibody.				cerol and less than		
	For a	a carrier free (BSA	and azide free) v	ersion of this product se	e product #24533.		
Specificity / Sensitivity		Histone H2A.X (D17A3) XP <sup>®</sup> Rabbit mAb recognizes endogenous levels of total histone H2A.X protein. This antibody does not cross-react with other histone H2A proteins.					
Source / Purificati	on Mon resid	Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues surrounding Val124 of human histone H2A.X protein.					

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Background	Histone H2A.X is a variant histone that represents approximately 10% of the total H2A histone proteins in normal human fibroblasts (1). H2A.X is required for checkpoint-mediated cell cycle arrest and DNA repair following double-stranded DNA breaks (1). DNA damage, caused by ionizing radiation, UV-light, or radiomimetic agents, results in rapid phosphorylation of H2A.X at Ser139 by PI3K-like kinases, including ATM, ATR, and DNA-PK (2,3). Within minutes following DNA damage, H2A.X is phosphorylated at Ser139 at sites of DNA damage (4). This very early event in the DNA-damage response is required for recruitment of a multitude of DNA-damage response proteins, including MDC1, NBS1, RAD50, MRE11, 53BP1, and BRCA1 (1). In addition to its role in DNA-damage repair, H2A.X is required for DNA fragmentation during apoptosis and is phosphorylated by various kinases in response to apoptotic signals. H2A.X is phosphorylated at Ser139 by DNA-PK in response to cell death receptor activation, c-Jun N-terminal Kinase (JNK1) in response to UV-A irradiation, and p38 MAPK in response to serum starvation (5-8). H2A.X is constitutively phosphorylated on Tyr142 in undamaged cells by WSTF (Williams-Beuren syndrome transcription factor) (9,10). Upon DNA damage, and concurrent with phosphorylation of Ser139, Tyr142 is dephosphorylated at sites of DNA damage to DNA repair proteins and apoptotic proteins to sites of DNA damage, phosphorylation at Tyr142 appears to determine which set of proteins are recruited. Phosphorylation of H2A.X at Tyr142 inhibits the recruitment of DNA repair proteins and poptotic inster so the sophorylation of H2A.X at Tyr142 phosphorylation of H2A.X at Tyr142 phosphorylation at Ser139 facilitates the recruitment of DNA repair proteins and poptotic proteins to sites of DNA damage, phosphorylation at Tyr142 appears to determine which set of proteins and promotes binding of pro-apoptotic factors such as JNK1 (9). Mouse embryonic fibroblasts expressing only mutant H2A.X Y142F, which favors recruitment of DNA repair p
Background Referer	1. Yuan, J. et al. (2010) FEBS Lett 584, 3717-24.         2. Rogakou, E.P. et al. (1998) J Biol Chem 273, 5858-68.         3. Burma, S. et al. (2001) J Biol Chem 276, 42462-7.         4. Rogakou, E.P. et al. (1999) J Cell Biol 146, 905-16.         5. Mukherjee, B. et al. (2006) DNA Repair (Amst) 5, 575-90.         6. Solier, S. et al. (2009) Mol Cell Biol 29, 68-82.         7. Lu, C. et al. (2006) Mol Cell 23, 121-32.         8. Lu, C. et al. (2008) FEBS Lett 582, 2703-8.         9. Cook, P.J. et al. (2009) Nature 458, 591-6.         10. Xiao, A. et al. (2009) Nature 457, 57-62.
Species Reactivity	Species reactivity is determined by testing in at least one approved application (e.g., western blot).
Western Blot Buffer	IMPORTANT: For western blots, incubate membrane with diluted primary antibody in 5% w/v BSA, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
Applications Key	WB: Western Blotting W-S: Simple Western™ IHC-P: Immunohistochemistry (Paraffin) IF-IC: Immunofluorescence (Immunocytochemistry) FC-FP: Flow Cytometry (Fixed/Permeabilized)
Cross-Reactivity Ke	<ul> <li>H: human M: mouse R: rat Hm: hamster Mk: monkey Vir: virus Mi: mink C: chicken Dm: D. melanogaster</li> <li>X: Xenopus Z: zebrafish B: bovine Dg: dog Pg: pig Sc: S. cerevisiae Ce: C. elegans Hr: horse</li> <li>GP: Guinea Pig Rab: rabbit All: all species expected</li> </ul>
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