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## Ubiquityl-Histone H2A (Lys119) (D27C4) XP<sup>®</sup> Rabbit mAb

For Research Use Only. Not for Use in Diagnostic Procedures.

Applications:	Reactivity:	Sensitivity:	MW (kDa):	Source/Isotype:	UniProt ID:	Entrez-Gene Id:
W, IP, IF-IC, FC-FP, ChIP, ChIP-seq, C&R, C&T	H M R Mk	Endogenous	23	Rabbit IgG	#Q96QV6	221613

### Product Usage Information

For optimal ChIP and ChIP-seq results, use 5 µl of antibody and 10 µg of chromatin (approximately 4 × 10<sup>6</sup> cells) per IP. This antibody has been validated using SimpleChIP<sup>®</sup> Enzymatic Chromatin IP Kits.

The CUT&RUN dilution was determined using CUT&RUN Assay Kit #86652.

The CUT&Tag dilution was determined using CUT&Tag Assay Kit #77552.

Application	Dilution
Western Blotting	1:2000
Immunoprecipitation	1:100
Immunofluorescence (Immunocytochemistry)	1:800 - 1:1600
Flow Cytometry (Fixed/Permeabilized)	1:800 - 1:3200
Chromatin IP	1:100
Chromatin IP-seq	1:100
CUT&RUN	1:50
CUT&Tag	1:50

### 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.

For a carrier free (BSA and azide free) version of this product see product #45772.

### Specificity/Sensitivity

Ubiquityl-Histone H2A (Lys119) (D27C4) XP<sup>®</sup> Rabbit mAb recognizes endogenous levels of histone H2A protein only when ubiquitinated at Lys119. The antibody does not cross-react with other ubiquitinated proteins or free ubiquitin.

### Source / Purification

Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the carboxy terminus of human histone H2A protein in which Lys119 is mono-ubiquitinated.

### Background

The nucleosome, made up of four core histone proteins (H2A, H2B, H3, and H4), is the primary building block of chromatin. Originally thought to function as a static scaffold for DNA packaging, histones have now been shown to be dynamic proteins, undergoing multiple types of posttranslational modifications, including acetylation, phosphorylation, methylation, and ubiquitination (1). Ubiquitin is a conserved 76 amino acid peptide unit that can be covalently linked to many cellular proteins by the ubiquitination process. Three components are involved in this protein-ubiquitin conjugation process. Ubiquitin is first activated by forming a thioester complex with the activation component E1; the activated ubiquitin is subsequently transferred to the ubiquitin-carrier protein E2, then from E2 to ubiquitin ligase E3 for final delivery to the epsilon-NH<sub>2</sub> of the target protein lysine residue (2). Histone H2A is mono-ubiquitinated at Lys119 by the Polycomb Repressor Complex 1 (PRC1) and is critical for transcriptional silencing of the developmental *HOX* genes and X chromosome inactivation (3-6). PRC1 is composed of Bmi1 and RING1A (also RING1 or RNF1), both of which act to enhance the E3 ubiquitin ligase activity of the catalytic subunit RING1B (also RING2 or RNF2) (3,4). Histone H2A is also mono-ubiquitinated at sites of DNA damage. This mono-ubiquitination event requires the PRC1 components Bmi1 and RING1B, in addition to another E3 ubiquitin ligase RNF8, and contributes to subsequent recruitment of the BRCA1 complex, via binding of RAP80/UIMC1 (ubiquitin interactive motif containing 1 protein) (7-10).

### Background References

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<b>Species Reactivity</b>	Species reactivity is determined by testing in at least one approved application (e.g., western blot).
<b>Western Blot Buffer</b>	<b>IMPORTANT:</b> 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.
<b>Applications Key</b>	<b>W:</b> Western Blotting <b>IP:</b> Immunoprecipitation <b>IF-IC:</b> Immunofluorescence (Immunocytochemistry) <b>FC-FP:</b> Flow Cytometry (Fixed/Permeabilized) <b>ChIP:</b> Chromatin IP <b>ChIP-seq:</b> Chromatin IP-seq <b>C&amp;R:</b> CUT&RUN <b>C&amp;T:</b> CUT&Tag
<b>Cross-Reactivity Key</b>	<b>H:</b> Human <b>M:</b> Mouse <b>R:</b> Rat <b>Mk:</b> Monkey
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