



Rabbit Anti-Phospho-Histone H2A.X (Tyr143) antibody

SL5360R

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| Product Name: | Phospho-Histone H2A.X (Tyr143) |
| Chinese Name: | 磷酸化组蛋白H2AX抗体 |
| Alias: | Histone H2A.X (phospho T143); Histone H2A.X (phospho Thr143); p-Histone H2A.X (T143); p-Histone H2A.X (Thr143); H2A histone family member X; Histone H2A.x; H2afx; H2a.x; H2ax; Hist5-2ax; H2A.X; H2A/X; H2AX; H2AFX; H2AX_HUMAN. |
| Organism Species: | Rabbit |
| Clonality: | Polyclonal |
| React Species: | Human,Mouse,Rat,Dog,Pig,Cow,Horse,Rabbit, |
| Applications: | ELISA=1:500-1000IHC-P=1:400-800IHC-F=1:400-800Flow-Cyt=1ug/TestICC=1:100-500IF=1:100-500 (Paraffin sections need antigen repair) not yet tested in other applications. optimal dilutions/concentrations should be determined by the end user. |
| Molecular weight: | 16kDa |
| Cellular localization: | The nucleus |
| Form: | Lyophilized or Liquid |
| Concentration: | 1mg/ml |
| immunogen: | KLH conjugated Synthesised phosphopeptide derived from human Histone H2AX around the phosphorylation site of Tyr143:QE(p-Y)-NH ₂ |
| Lsotype: | IgG |
| Purification: | affinity purified by Protein A |
| Storage Buffer: | 0.01M TBS(pH7.4) with 1% BSA, 0.03% Proclin300 and 50% Glycerol. |
| Storage: | Store at -20 °C for one year. Avoid repeated freeze/thaw cycles. The lyophilized antibody is stable at room temperature for at least one month and for greater than a year when kept at -20°C. When reconstituted in sterile pH 7.4 0.01M PBS or diluent of antibody the antibody is stable for at least two weeks at 2-4 °C. |
| PubMed: | PubMed |
| Product Detail: | Histones are basic nuclear proteins that are responsible for the nucleosome structure of |

the chromosomal fiber in eukaryotes. Two molecules of each of the four core histones (H2A, H2B, H3, and H4) form an octamer, around which approximately 146 bp of DNA is wrapped in repeating units, called nucleosomes. The linker histone, H1, interacts with linker DNA between nucleosomes and functions in the compaction of chromatin into higher order structures. This gene encodes a member of the histone H2A family, and generates two transcripts through the use of the conserved stem-loop termination motif, and the polyA addition motif. [provided by RefSeq, Jul 2008].

Function:

Variant histone H2A which replaces conventional H2A in a subset of nucleosomes. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling. Required for checkpoint-mediated arrest of cell cycle progression in response to low doses of ionizing radiation and for efficient repair of DNA double strand breaks (DSBs) specifically when modified by C-terminal phosphorylation.

Subunit:

The nucleosome is a histone octamer containing two molecules each of H2A, H2B, H3 and H4 assembled in one H3-H4 heterotetramer and two H2A-H2B heterodimers. The octamer wraps approximately 147 bp of DNA. Interacts with numerous proteins required for DNA damage signaling and repair when phosphorylated on Ser-140. These include MDC1, TP53BP1, BRCA1 and the MRN complex, composed of MRE11A, RAD50, and NBN. Interaction with the MRN complex is mediated at least in part by NBN. Also interacts with DHX9/NDHII when phosphorylated on Ser-140 and MCPH1 when phosphorylated at Ser-140 or Tyr-143. Interacts with ARRB2; the interaction is detected in the nucleus upon OR1D2 stimulation.

Subcellular Location:

Nucleus. Chromosome.

Post-translational modifications:

Phosphorylated on Ser-140 (to form gamma-H2AFX or H2AX139ph) in response to DNA double strand breaks (DSBs) generated by exogenous genotoxic agents and by stalled replication forks, and may also occur during meiotic recombination events and immunoglobulin class switching in lymphocytes. Phosphorylation can extend up to several thousand nucleosomes from the actual site of the DSB and may mark the surrounding chromatin for recruitment of proteins required for DNA damage signaling and repair. Widespread phosphorylation may also serve to amplify the damage signal or aid repair of persistent lesions. Phosphorylation of Ser-140 (H2AX139ph) in response to ionizing radiation is mediated by both ATM and PRKDC while defects in DNA replication induce Ser-140 phosphorylation (H2AX139ph) subsequent to activation of ATR and PRKDC. Dephosphorylation of Ser-140 by PP2A is required for DNA DSB repair. In meiosis, Ser-140 phosphorylation (H2AX139ph) may occur at synaptonemal

complexes during leptotene as an ATM-dependent response to the formation of programmed DSBs by SPO11. Ser-140 phosphorylation (H2AX139ph) may subsequently occur at unsynapsed regions of both autosomes and the XY bivalent during zygotene, downstream of ATR and BRCA1 activation. Ser-140 phosphorylation (H2AX139ph) may also be required for transcriptional repression of unsynapsed chromatin and meiotic sex chromosome inactivation (MSCI), whereby the X and Y chromosomes condense in pachytene to form the heterochromatic XY-body. During immunoglobulin class switch recombination in lymphocytes, Ser-140 phosphorylation (H2AX139ph) may occur at sites of DNA-recombination subsequent to activation of the activation-induced cytidine deaminase AICDA. Phosphorylation at Tyr-143 (H2AXY142ph) by BAZ1B/WSTF determines the relative recruitment of either DNA repair or pro-apoptotic factors. Phosphorylation at Tyr-143 (H2AXY142ph) favors the recruitment of APBB1/FE65 and pro-apoptosis factors such as MAPK8/JNK1, triggering apoptosis. In contrast, dephosphorylation of Tyr-143 by EYA proteins (EYA1, EYA2, EYA3 or EYA4) favors the recruitment of MDC1-containing DNA repair complexes to the tail of phosphorylated Ser-140 (H2AX139ph). Monoubiquitination of Lys-120 (H2AXK119ub) by RING1 and RNF2/RING2 complex gives a specific tag for epigenetic transcriptional repression (By similarity). Following DNA double-strand breaks (DSBs), it is ubiquitinated through 'Lys-63' linkage of ubiquitin moieties by the E2 ligase UBE2N and the E3 ligases RNF8 and RNF168, leading to the recruitment of repair proteins to sites of DNA damage. Ubiquitination at Lys-14 and Lys-16 (H2AK13Ub and H2AK15Ub, respectively) in response to DNA damage is initiated by RNF168 that mediates monoubiquitination at these 2 sites, and 'Lys-63'-linked ubiquitin are then conjugated to monoubiquitin; RNF8 is able to extend 'Lys-63'-linked ubiquitin chains in vitro. H2AK119Ub and ionizing radiation-induced 'Lys-63'-linked ubiquitination (H2AK13Ub and H2AK15Ub) are distinct events. Acetylation at Lys-37 increases in S and G2 phases. This modification has been proposed to play a role in DNA double-strand break repair.

Similarity:

Belongs to the histone H2A family.

SWISS:

P16104

Gene ID:

3014

Database links:

[Entrez Gene: 3014](#)Human

[Entrez Gene: 15270](#)Mouse

[Entrez Gene: 500987](#)Rat

[Omim: 601772](#)Human

[SwissProt: P16104](#)Human

[SwissProt: P27661](#)Mouse

[Unigene: 477879](#)Human

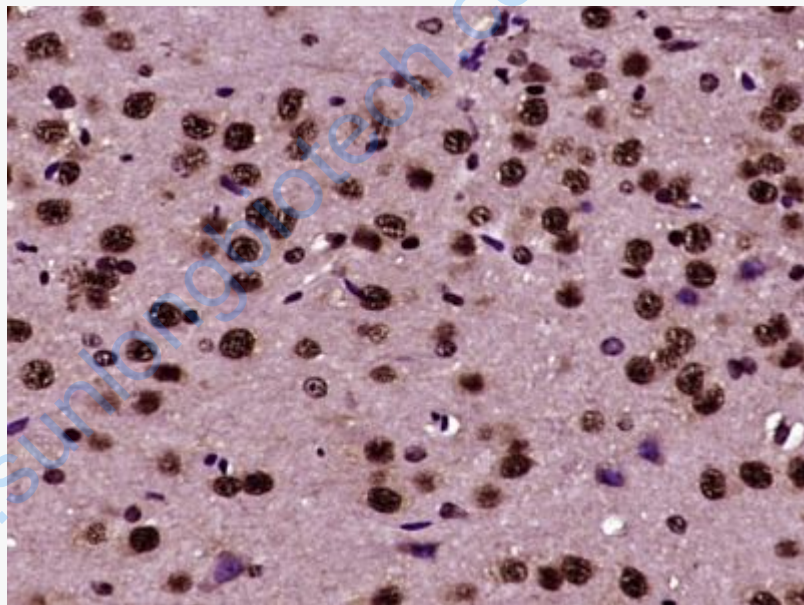
[Unigene: 245931](#)Mouse

[Unigene: 2850](#)Rat

Important Note:

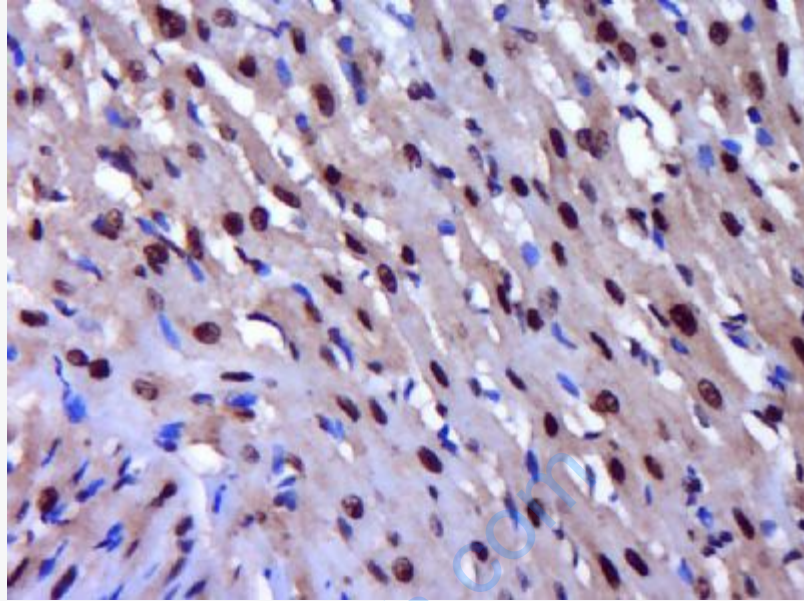
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H2AX蛋白属于组蛋白一族, 组蛋白参与细胞内DNA的组合、包装.

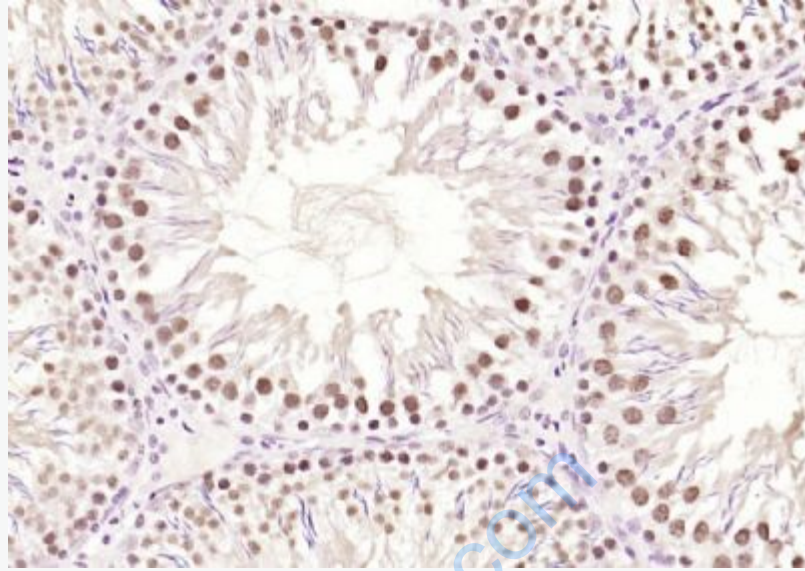


Picture:

Paraformaldehyde-fixed, paraffin embedded (Mouse brain); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-Histone H2A.X (Tyr143)) Polyclonal Antibody, Unconjugated (SL5360R) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructions and DAB staining.

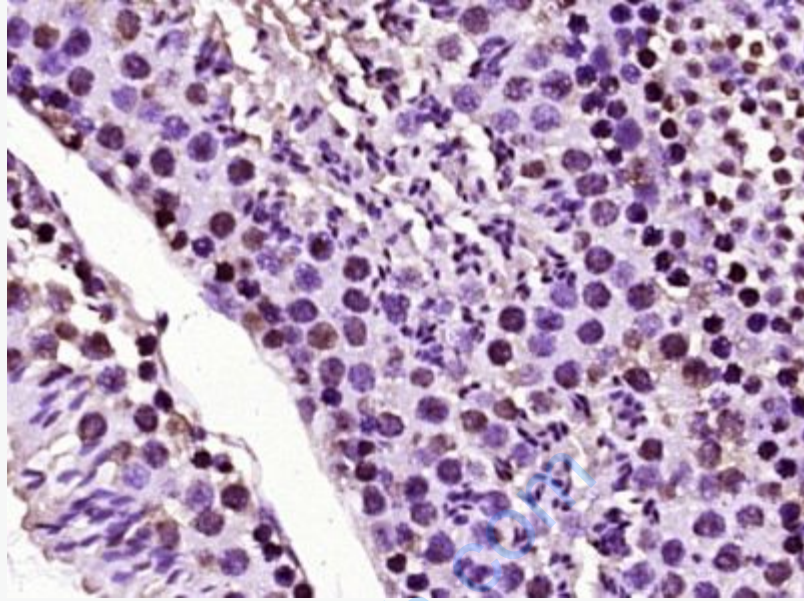


Paraformaldehyde-fixed, paraffin embedded (Rat heart); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (H2AX) Polyclonal Antibody, Unconjugated (SL5360R) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructions and DAB staining.

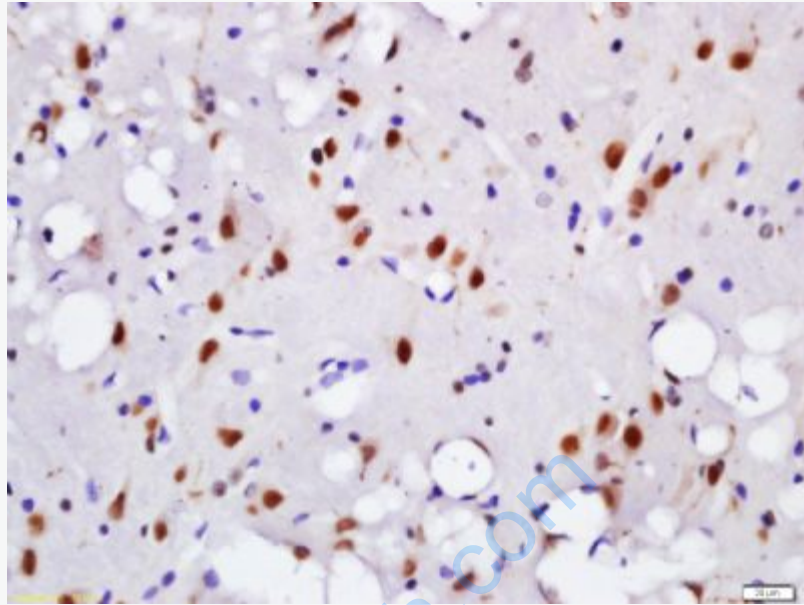


Paraformaldehyde-fixed, paraffin embedded (rat testis); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-Histone H2A.X (Tyr143)) Polyclonal Antibody, Unconjugated (SL5360R) at 1:200 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructions and DAB staining.

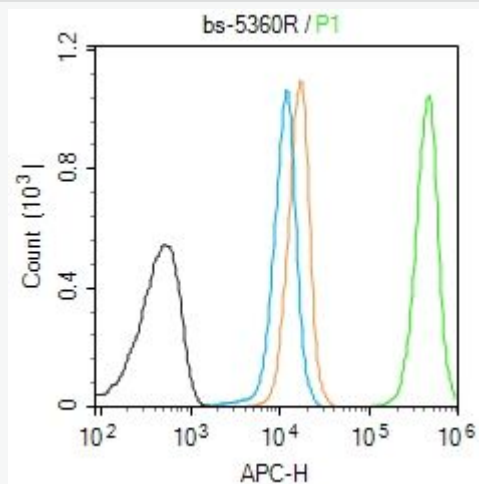
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Paraformaldehyde-fixed, paraffin embedded (Mouse testis); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (Phospho-Histone H2A.X (Tyr143)) Polyclonal Antibody, Unconjugated (SL5360R) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructions and DAB staining.



Tissue/cell: rat brain tissue; 4% Paraformaldehyde-fixed and paraffin-embedded;
Antigen retrieval: citrate buffer (0.01M, pH 6.0), Boiling bathing for 15min; Block endogenous peroxidase by 3% Hydrogen peroxide for 30min; Blocking buffer (normal goat serum,C-0005) at 37°C for 20 min;
Incubation: Anti-Phospho-Histone H2A.X(Tyr143) Polyclonal Antibody, Unconjugated(SL5360R) 1:200, overnight at 4°C, followed by conjugation to the secondary antibody(SP-0023) and DAB(C-0010) staining



Blank control (Black line): Molt4 (Black).

Primary Antibody (green line): Rabbit Anti-Phospho-Histone H2A.X (Tyr143) antibody (SL5360R)

Dilution: $1\mu\text{g} / 10^6$ cells;

Isotype Control Antibody (orange line): Rabbit IgG .

Secondary Antibody (white blue line): Goat anti-rabbit IgG-AF647

Dilution: $1\mu\text{g} / \text{test}$.

Protocol

The cells were fixed with 4% PFA (10min at room temperature) and then permeabilized with 90% ice-cold methanol for 20 min at room temperature. The cells were then incubated in 5% BSA to block non-specific protein-protein interactions for 30 min at room temperature. Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.