

## Anti-Histone H2A.X/H2AFX Antibody Picoband®

Catalog Number: A00241-1

### About H2AFX

H2A histone family member X (usually abbreviated asH2AX) is a type of histone protein from the H2A family encoded by the H2AFX gene. 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 replication-independent histone that is 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.

### Overview

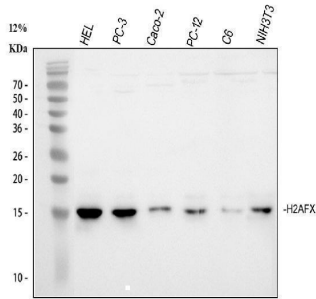
Product Name	Anti-Histone H2A.X/H2AFX Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Histone H2A.X/H2AFX Antibody Picoband® catalog # A00241-1. Tested in ELISA, Flow Cytometry, IP, IHC, WB applications. This antibody reacts with Human, Mouse, Rat. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	ELISA, Flow Cytometry, IP, IHC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na <sub>2</sub> HPO <sub>4</sub> .
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	P16104

### Technical Details

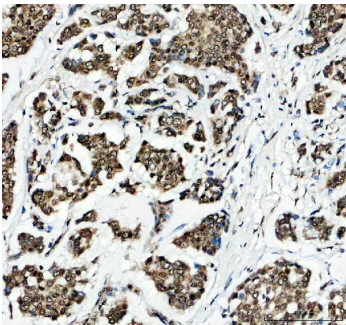
Immunogen	E.coli-derived human Histone H2A.X/H2AFX recombinant protein (Position: S2-T121).
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P).
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized

Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human, Mouse Immunoprecipitation, 0.5-2 ug/ml, Human Flow Cytometry (Fixed), 1-3ug/1x10 <sup>6</sup> cells, Human ELISA, 0.1-0.5ug/ml, -

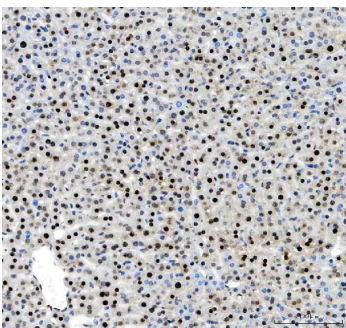
## Anti-Histone H2A.X/H2AFX Antibody Picoband® (A00241-1) Images



Western blot analysis of H2AFX using anti-H2AFX antibody (A00241-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human HEL whole cell lysates, Lane 2: human PC-3 whole cell lysates, Lane 3: human CACO-2 whole cell lysates, Lane 4: rat PC-12 whole cell lysates, Lane 5: rat C6 whole cell lysates, Lane 6: mouse NIH/3T3 whole cell lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-H2AFX antigen affinity purified polyclonal antibody (Catalog # A00241-1) at 0.5 ug/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for H2AFX at approximately 15 kDa. The expected band size for H2AFX is at 15 kDa.

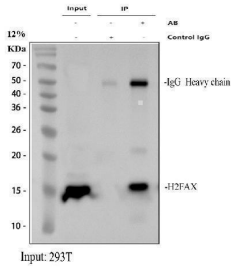


IHC analysis of H2AFX using anti-H2AFX antibody (A00241-1). H2AFX was detected in a paraffin-embedded section of human breast cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-H2AFX Antibody (A00241-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

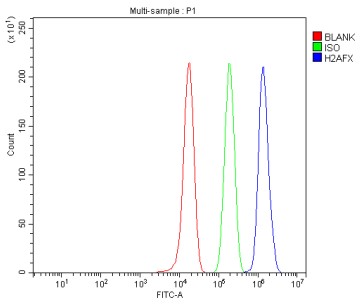


IHC analysis of H2AFX using anti-H2AFX antibody (A00241-1). H2AFX was detected in a paraffin-embedded section of mouse liver tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-H2AFX Antibody (A00241-1) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

Immunoprecipitating (IP) H2AFX in 293T whole cell lysate. Western blot analysis of H2AFX using anti-H2AFX antibody (A00241-1); Lane 1: 293T whole cell lysates (30ug); Lane 2:



Rabbit control IgG instead of anti-H2AFX antibody in 293T whole cell lysate; Lane 3: anti-H2AFX antibody (2ug) + 293T whole cell lysate (500ug). After electrophoresis, proteins were transferred to a membrane. Then the membrane was incubated with rabbit anti-H2AFX antigen affinity purified polyclonal antibody (A00241-1) at a dilution of 0.5 ug/mL and probed with a goat anti-rabbit IgG-HRP secondary antibody (Catalog # BA1054). The signal is developed using ECL Plus Western Blotting Substrate (Catalog # AR1196-200). A specific band was detected for H2AFX at approximately 15 kDa. The expected band size for H2AFX is at 15 kDa.



Flow Cytometry analysis of PC-3 cells using anti-H2AFX antibody (A00241-1). Overlay histogram showing PC-3 cells stained with A00241-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-H2AFX Antibody (A00241-1, 1 ug/1x10<sup>6</sup> cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10<sup>6</sup> cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10<sup>6</sup>) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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### Anti-Histone H2A.X/H2AFX Antibody

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