

Anti-Histone H2A.X antibody - ChIP Grade ab20669

★★★★★ [7 Abreviews](#) [29 References](#) [8 图像](#)

概述

产品名称	Anti-Histone H2A.X抗体- ChIP Grade
描述	兔多克隆抗体to Histone H2A.X - ChIP Grade
宿主	Rabbit
经测试应用	适用于: ChIP, WB, IP, ICC/IF, IHC-P
种属反应性	与反应: Mouse, Rat, Human
免疫原	Synthetic peptide corresponding to Human Histone H2A.X aa 100 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. (Peptide available as ab15646)

常规说明

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS
纯度	Immunogen affinity purified
克隆	多克隆
同种型	IgG

应用

The Abpromise guarantee **Abpromise™**承诺保证使用ab20669于以下的经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ChIP		Use 2 µg for 25 µg of chromatin.
WB	★★★★★ (3)	Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).
IP		Use at an assay dependent concentration.
ICC/IF	★★★★★ (4)	Use at an assay dependent concentration.
IHC-P		Use a concentration of 0.2 - 0.5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

靶标

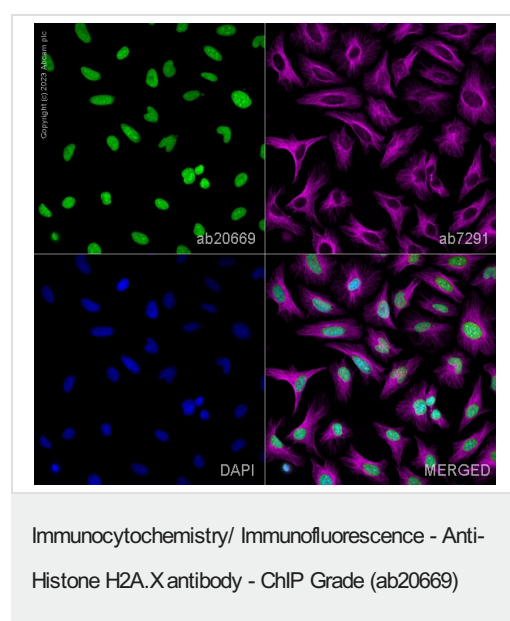
功能	Variant histone H2A which replaces conventional H2A in a subset of nucleosomes. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA 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.
序列相似性	Belongs to the histone H2A family.
发展阶段	Synthesized in G1 as well as in S-phase.
结构域	The [ST]-Q motif constitutes a recognition sequence for kinases from the PI3/PI4-kinase family.
翻译后修饰	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 occurs 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. 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. Monoubiquitination and ionizing radiation-induced 'Lys-63'-linked ubiquitination are distinct events.

细胞定位

Nucleus. Chromosome.

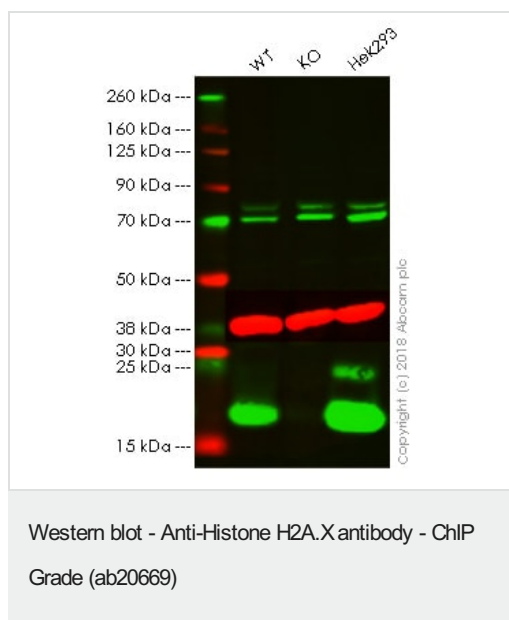
图片



ab20669 staining Histone H2A.X in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab20669 at 0.1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



All lanes : Anti-Histone H2A.X antibody - ChIP Grade (ab20669) at 1 µg/ml

Lane 1 : Wild-type HAP1 whole cell lysate

Lane 2 : H2AFX (Histone H2A.X) knockout HAP1 whole cell lysate

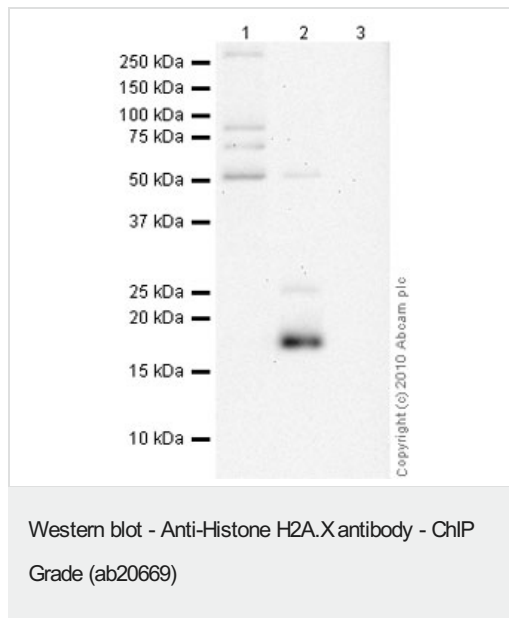
Lane 3 : HEK 293 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 15 kDa

Lanes 1 - 3: Merged signal (red and green). Green - ab20669 observed at 17 kDa. Red - loading control, **ab9484**, observed at 37 kDa.

ab20669 was shown to recognize Histone H2A.X in wild-type HAP1 cells as signal was lost at the expected MW in H2AFX (Histone H2A.X) knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and H2AFX (Histone H2A.X) knockout samples were subjected to SDS-PAGE. Ab20669 and **ab9484** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/ml and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



All lanes : Anti-Histone H2A.X antibody - ChIP Grade (ab20669) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Nuclear Lysate at 10 µg

Lane 2 : HeLa Histone Preparation Nuclear Lysate at 2.5 µg

Lane 3 : Histone H2A Recombinant Protein (negative control) at 0.1 µg

Secondary

All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) ([ab65484](#)) at 1/3000 dilution

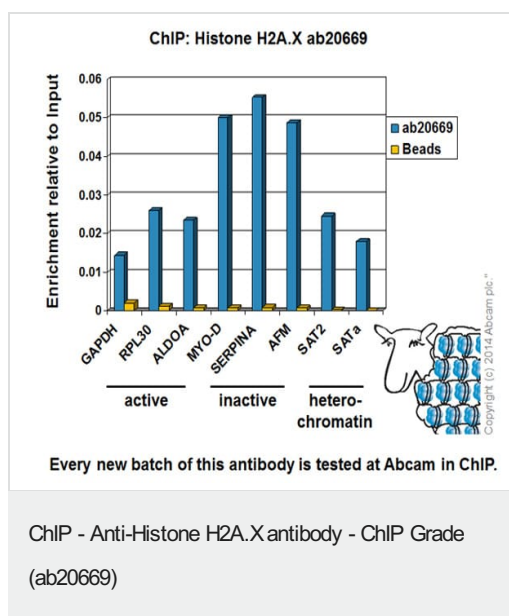
Developed using the ECL technique.

Performed under reducing conditions.

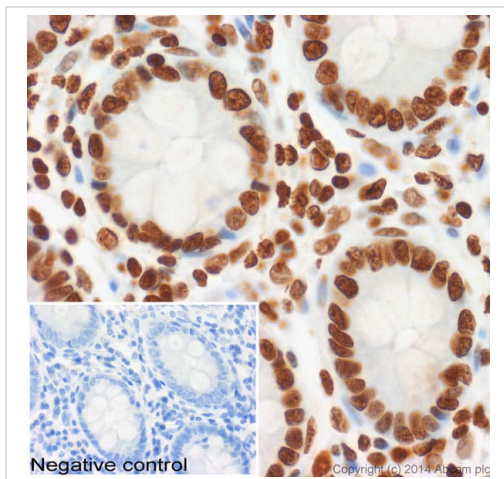
Predicted band size: 15 kDa

Observed band size: 17 kDa

Exposure time: 20 minutes



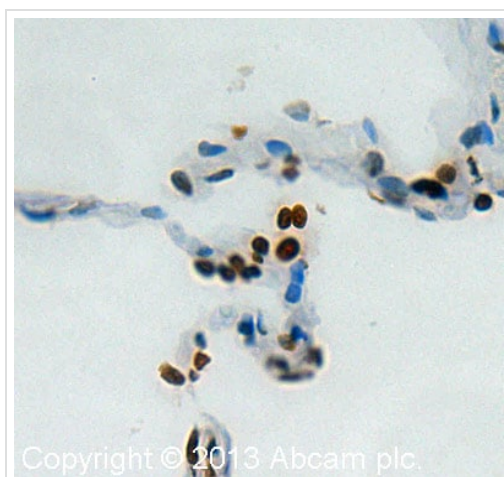
Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of ab20669 (blue), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are located in the first kb of the transcribed region.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody - ChIP Grade (ab20669)

IHC image of ab20669 staining Histone H2A.X in human colon formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab20669, 0.5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the negative control (shown on the inset).

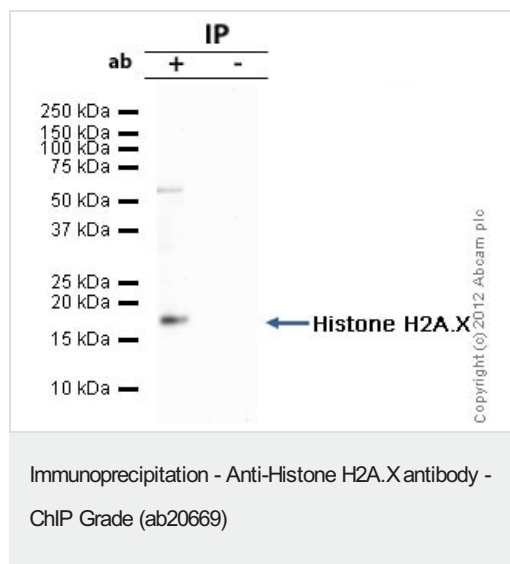
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody - ChIP Grade (ab20669)

IHC image of Histone H2A.X staining in Human Lung formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab20669, 0.2 µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



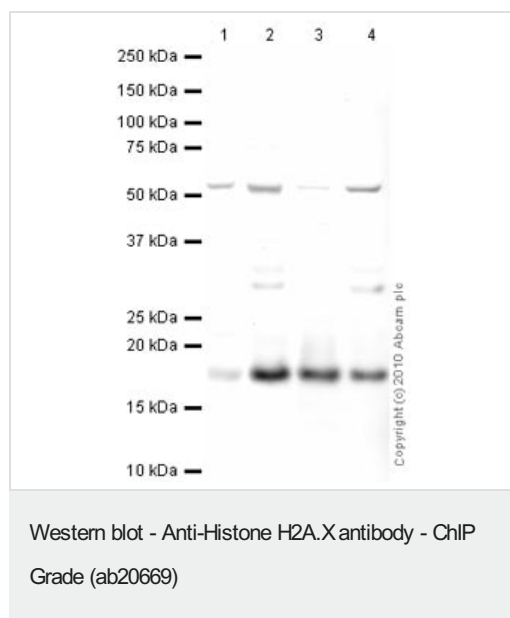
Histone H2A.X was immunoprecipitated using 0.5mg Rat Testis whole tissue extract, 5µg of Rabbit polyclonal to Histone H2A.X and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Rat Testis whole tissue extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab20669.

Secondary: Clean blot (HRP conjugate) at 1/1000 dilution.

Band: 17kDa: Histone H2A.X.



All lanes : Anti-Histone H2A.X antibody - ChIP Grade (ab20669) at 1 µg/ml

Lane 1 : Lung (Mouse) Tissue Lysate

Lane 2 : Testis (Mouse) Tissue Lysate

Lane 3 : Lung (Rat) Tissue Lysate

Lane 4 : Testis (Rat) Tissue Lysate - normal tissue ([ab29388](#))

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa

Observed band size: 17 kDa

Additional bands at: 55 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 8 minutes

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