abcam

Product datasheet

# Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade ab52484

重组

11 Abreviews 42 References 13 图像

## 概述

| | |
|---|---|
| **产品名称** | Anti-Histone H2B抗体[mAbcam 52484] - ChIP Grade |
| **描述** | 小鼠单克隆抗体[mAbcam 52484] to Histone H2B - ChIP Grade |
| **宿主** | Mouse |
| **经测试应用** | **适用于:** IP, WB, ICC/IF, ChIP, IHC-P, Flow Cyt (Intra) |
| **种属反应性** | **与反应:** Mouse, Rat, Human<br>**预测可用于:** Chicken, Cow, Xenopus laevis, Caenorhabditis elegans, Drosophila melanogaster, Zebrafish, Orangutan |
| **免疫原** | Synthetic peptide corresponding to Human Histone H2B aa 100 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. |
| **阳性对照** | WB: HeLa, 293T, MEF, PC-12, Human heart and mouse heart tissue lysates (zebrafish brain, heart, liver and skeletal muscle homogenates) IHC-P: Human breast cancer tissue, mouse lung, rat lung ICC/IF: HeLa cells, NIH/3T3 cells |
| **常规说明** | This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**.<br>This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**. |

## 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** | Preservative: 0.01% Sodium azide<br>Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA |

| | |
|---|---|
| **纯度** | Protein A purified |
| **克隆** | 单克隆 |
| **克隆编号** | mAbcam 52484 |
| **骨髓瘤** | Sp2/0 |
| **同种型** | IgG1 |

## 应用

**The Abpromise guarantee** Abpromise™承诺保证使用ab52484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 |
|---|---|---|
| **IP** | | 1/30. |
| **WB** | ★★★★★ (3) | 1/1000. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa). |
| **ICC/IF** | ★★★★☆ (3) | 1/2000. |
| **ChIP** | ★★★★★ (2) | Use 2 µg for 25 µg of chromatin. |
| **IHC-P** | ★★★★★ (1) | 1/20000 - 1/50000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. |
| **Flow Cyt (Intra)** | | 1/100.<br>**ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody. |

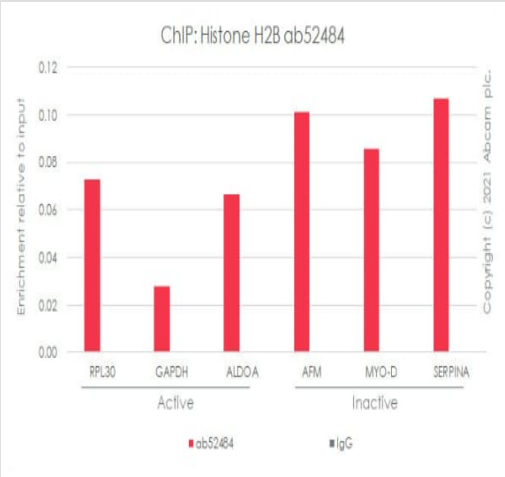
## 靶标

**相关性** Core component of nucleosome. 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. Subunit structure 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. Post-translational modification Monoubiquitination at Lys-35 (H2BK34Ub) by the MSL1/MSL2 dimer is required for histone H3 'Lys-4' (H3K4me) and 'Lys-79' (H3K79me) methylation and transcription activation at specific gene loci, such as HOXA9 and MEIS1 loci. Similarly, monoubiquitination at Lys-121 (H2BK120Ub) by the RNF20/40 complex gives a specific tag for epigenetic transcriptional activation and is also prerequisite for histone H3 'Lys-4' and 'Lys-79' methylation. It also functions cooperatively with the FACT dimer to stimulate elongation by RNA polymerase II. H2BK120Ub also acts as a regulator of mRNA splicing: deubiquitination by USP49 is required for efficient cotranscriptional splicing of a large set of exons. Phosphorylation at Ser-37 (H2BS36ph) by AMPK in response to stress promotes transcription. Phosphorylated on Ser-15 (H2BS14ph) by STK4/MST1 during apoptosis; which

facilitates apoptotic chromatin condensation. Also phosphorylated on Ser-15 in response to DNA double strand breaks (DSBs), and in correlation with somatic hypermutation and immunoglobulin class-switch recombination. GlcNAcylation at Ser-113 promotes monoubiquitination of Lys-121. It fluctuates in response to extracellular glucose, and associates with transcribed genes. Crotonylation (Kcr) is specifically present in male germ cells and marks testis-specific genes in post-meiotic cells, including X-linked genes that escape sex chromosome inactivation in haploid cells. Crotonylation marks active promoters and enhancers and confers resistance to transcriptional repressors. It is also associated with post-meiotically activated genes on autosomes.

**细胞定位** Nuclear

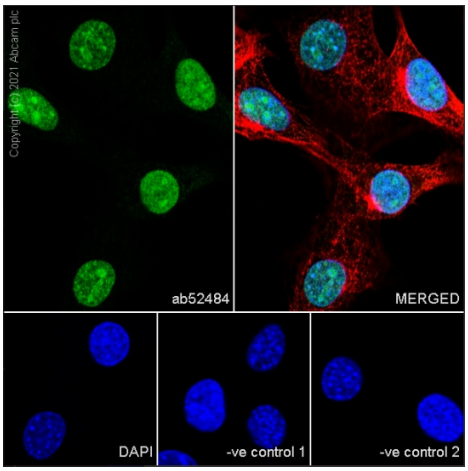
## 图片




ChIP - Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484)

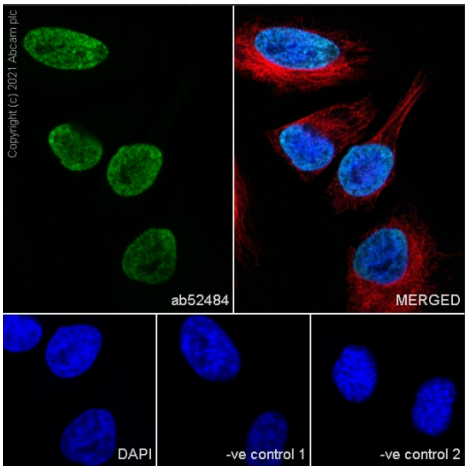
Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol*. Cells were fixed with formaldehyde for 10min.

The ChIP was performed with 25 µg of chromatin, 2 µg of ab52484 (red), or 2 µg of mouse IgG1 **ab18443** (gray) and 25 µl of Protein A/G Dynabeads. The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).



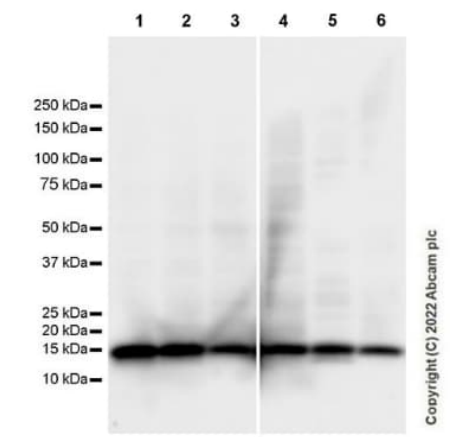

Immunocytochemistry - Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484)

NIH/3T3 cells were fixed in 100% methanol and permeabilized with 0.1% Triton X-100. Primary antibody, ab52484 at 1:2000 was incubated overnight at 4° C, followed by AlexaFluor® 488-conjugated Goat anti-mouse secondary antibody (**ab150117**) at 1/1000 dilution at RT for 45 min. **ab179513** Anti-beta Tubulin, used as a counterstain at 1/200 dilution, was co-incubated with ab52484 overnight at 4° C, followed by Alexa Fluor® 594 Goat Anti-Rabbit secondary (**ab150080**) at 1/1000 dilution at RT for 45 min. Nucleus were visualized using DAPI.




Immunocytochemistry/ Immunofluorescence - Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484)

HeLa cells were fixed in 100% methanol and permeabilized with 0.1% Triton X-100. Primary antibody, ab52484 at 1:2000 was incubated overnight at 4° C, followed by AlexaFluor® 488-conjugated Goat anti-mouse secondary antibody (**ab150117**) at 1/1000 dilution at RT for 45 min. **ab179513** Anti-beta Tubulin, used as a counterstain at 1/200 dilution, was co-incubated with ab52484 overnight at 4° C, followed by Alexa Fluor® 594 Goat Anti-Rabbit secondary (**ab150080**) at 1/1000 dilution at RT for 45 min. Nucleus were visualized using DAPI.




Western blot - Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484)

**All lanes :** Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484) at 1/1000 dilution

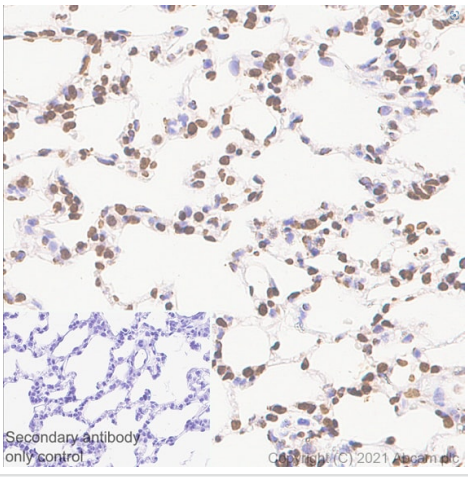
**Lane 1 :** HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
**Lane 2 :** 293T (human embryonic kidney epithelial cell), whole cell lysate
**Lane 3 :** MEF (mouse embryonic fibroblast (immortalized)), whole cell lysate
**Lane 4 :** PC-12 (rat adrenal gland pheochromocytoma), whole cell lysate
**Lane 5 :** Human heart tissue lysate
**Lane 6 :** Mouse heart tissue lysate

Lysates/proteins at 20 µg per lane.

**Secondary**
**All lanes :** Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/5000 dilution

**Predicted band size:** 15 kDa
**Observed band size:** 15 kDa

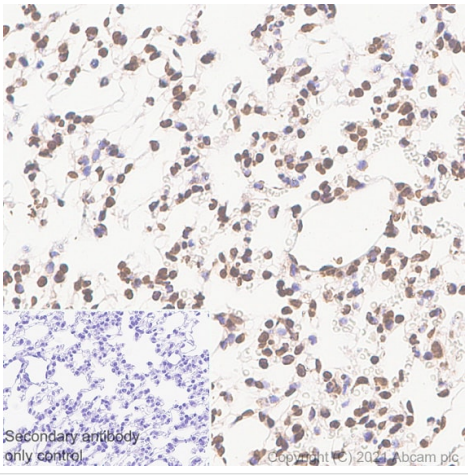
**Exposure time:** 3 seconds




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484)

Nuclear staining in rat lung. The section was heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins. The section was incubated with ab52484 (1:50000 (0.021 µg/ml)) for 10 mins at room temperature, followed by anti-mouse IgG1 antibody (**ab125913**) for 8 mins during the LeicaDS9800 kit staining procedure.

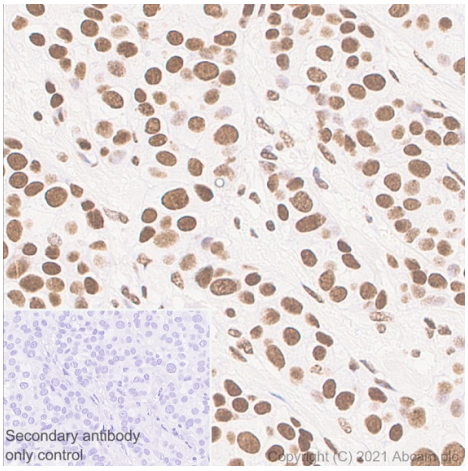
The immunostaining was performed on a Leica Biosystems BOND® RX instrument




Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484)

Nuclear staining in mouse lung. The section was heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins. The section was incubated with ab52484 (1:50000 (0.021 µg/ml)) for 10 mins at room temperature, followed by anti-mouse IgG1 antibody (**ab125913**) for 8 mins during the LeicaDS9800 kit staining procedure.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument



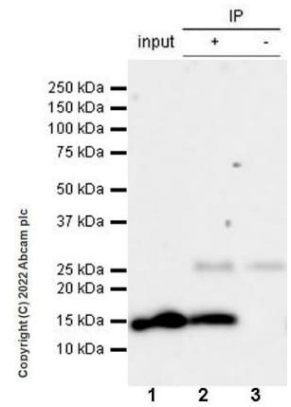

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484)

Nuclear staining in human breast cancer. The section was heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins.

The section was incubated with ab52484 at 1:20000 (0.053 µg/ml) for 10 mins at room temperature, followed by anti-mouse IgG1 antibody (**ab125913**) for 8 mins during the LeicaDS9800 kit staining procedure.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument


Immunoprecipitation - Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484)




Immunoprecipitation - Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484)

Histone H2B was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate with AB52484 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using AB52484 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

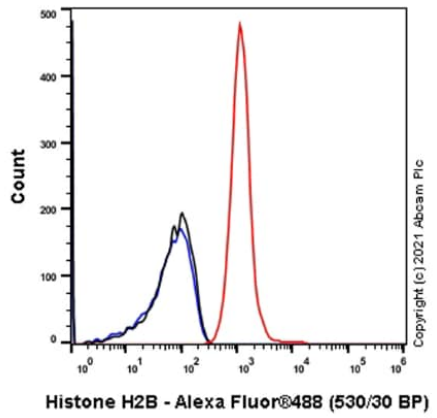
Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 ug

Lane 2: AB52484 IP in HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 3: Mouse monoclonal IgG (**ab18443**) instead of AB52484 in HeLa whole cell lysate

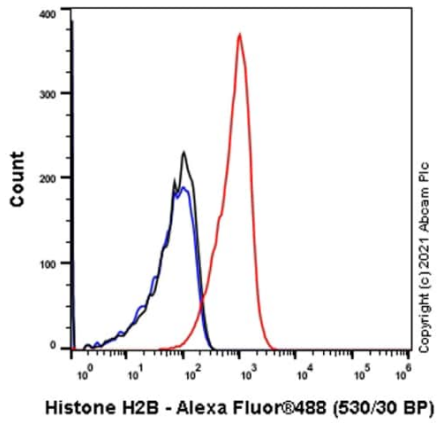
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 32 seconds.



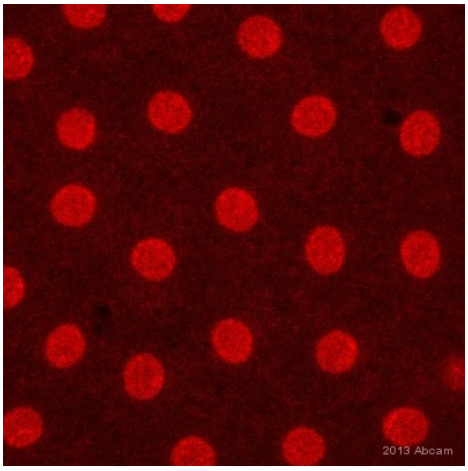

Flow cytometric analysis of NIH/3T3 (mouse embryonic fibroblast) cells labelling Histone H2B with AB52484 at 1/100 dilution (1 µg) (Red) compared with a Mouse monoclonal IgG (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti mouse IgG (Alexa Fluor® 488, **ab150113**) at 1/2000 dilution was used as the secondary antibody.

Flow Cytometry (Intracellular) - Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484)




Flow cytometric analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling Histone H2B with AB52484 at 1/1000 dilution (0.1µg) (Red) compared with a Mouse monoclonal IgG (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti mouse IgG (Alexa Fluor® 488, **ab150113**) at 1/2000 dilution was used as the secondary antibody.

Flow Cytometry (Intracellular) - Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484)

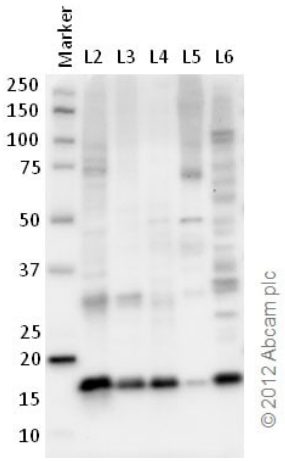



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484)

This image is courtesy of an anonymous Abreview

This image was generated using a previous batch manufactured using hybridoma production method.

ab52484 staining Histone H2B in Fruit fly (Drosophila melanogaster) embryo cells by ICC/IF (Immunocytochemistry/immunofluorescence). Embryos were washed in 1x PBS + 0.1% Trition, cells were fixed with heat, and blocked with 10% BSA for 12 hours at 4°C. Samples were incubated with primary antibody (1/1000) for 24 hours. An Alexa Fluor®594-conjugated Rabbit anti-mouse IgG polyclonal (1/5000) was used as the secondary antibody.




Western blot - Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484)

**All lanes :** Anti-Histone H2B antibody [mAbcam 52484] - ChIP Grade (ab52484) at 5 µg/ml

**Lane 1 :** Marker
**Lane 2 :** Zebrafish brain homogenate at 20 µg
**Lane 3 :** Zebrafish heart homogenate at 10 µg
**Lane 4 :** Zebrafish liver homogenate at 10 µg
**Lane 5 :** Zebrafish skeletal muscle homogenate at 10 µg
**Lane 6 :** HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate at 10 µg

**Secondary**
**All lanes :** Goat polyclonal to Mouse IgG – H&L – Pre-Adsorbed (HRP) at 1/6000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 15 kDa
**Observed band size:** 17 kDa

**Exposure time:** 1 minute

This image was generated using a
previous batch manufactured using hybridoma production method

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