

### Anti-mH2A1 antibody [14G7] ab91528

KO 評価済

★★★★☆ 1 Abreviews 画像数 5

#### 製品の概要

製品名	Anti-mH2A1 antibody [14G7]
製品の詳細	Mouse monoclonal [14G7] to mH2A1
由来種	Mouse
アプリケーション	<b>適用あり:</b> ChIP, WB, Flow Cyt (Intra), ICC/IF
種交差性	<b>交差種:</b> Human
免疫原	Recombinant full length protein corresponding to Human mH2A1.
ポジティブ・コントロール	This antibody gave a positive signal in the following whole cell lysates: HeLa; HepG2; MCF7
特記事項	<p>This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact <a href="mailto:orders@abcam.com">orders@abcam.com</a>.</p> <p>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.</p> <p>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&amp;As</p>

#### 製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid repeated freeze / thaw cycles.
バッファー	<p>pH: 7.40</p> <p>Preservative: 0.02% Sodium azide</p> <p>Constituent: PBS</p>
精製度	Protein G purified
ポリ/モノ	モノクローナル
クローン名	14G7
アイソタイプ	IgG2

## アプリケーション

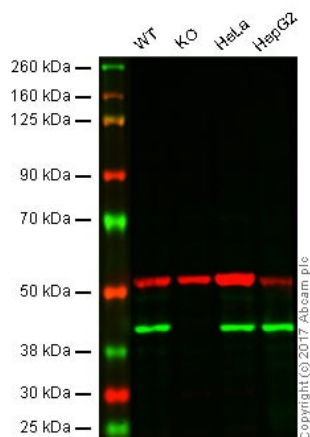
The Abpromise guarantee **Abpromise保証は、次のテスト済みアプリケーションにおけるab91528の使用に適用されます**  
 アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ChIP		Use at an assay dependent concentration.
WB		Use a concentration of 10 µg/ml. Detects a band of approximately 40 kDa (predicted molecular weight: 40 kDa).
Flow Cyt (Intra)		Use 1µg for 10 <sup>6</sup> cells. <b>ab170190</b> - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (1)	Use a concentration of 1 µg/ml.

## ターゲット情報

機能	Variant histone H2A which replaces conventional H2A in a subset of nucleosomes where it represses transcription. 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. Involved in stable X chromosome inactivation. Inhibits the binding of transcription factors and interferes with the activity of remodeling SWI/SNF complexes. Inhibits histone acetylation by EP300 and recruits class I HDACs, which induces an hypoacetylated state of chromatin. In addition, isoform 1, but not isoform 2, binds ADP-ribose and O-acetyl-ADP-ribose, and may be involved in ADP-ribose-mediated chromatin modulation.
組織特異性	Ubiquitous.
配列類似性	Contains 1 histone H2A domain. Contains 1 Macro domain.
翻訳後修飾	Monoubiquitinated at either Lys-116 or Lys-117. May also be polyubiquitinated. Ubiquitination is mediated by the CUL3/SPOP E3 complex and does not promote proteasomal degradation. Instead, it is required for enrichment in inactive X chromosome chromatin.
細胞内局在	Nucleus. Chromosome. Enriched in inactive X chromosome chromatin and in senescence-associated heterochromatin.

## 画像



Western blot - Anti-mH2A1 antibody [14G7] (ab91528)

**Lane 1:** Wild-type HAP1 whole cell lysate (20 µg)

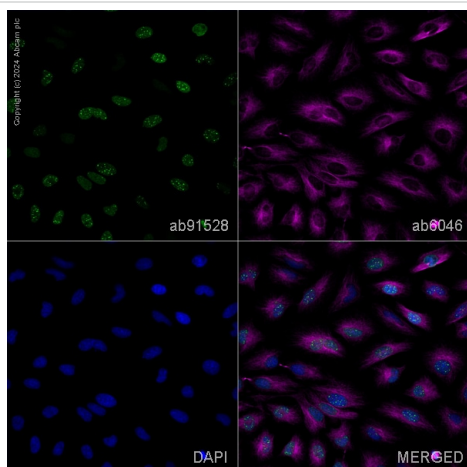
**Lane 2:** mH2A1 knockout HAP1 whole cell lysate (20 µg)

**Lane 3:** HeLa whole cell lysate (20 µg)

**Lane 4:** HepG2 whole cell lysate (20 µg)

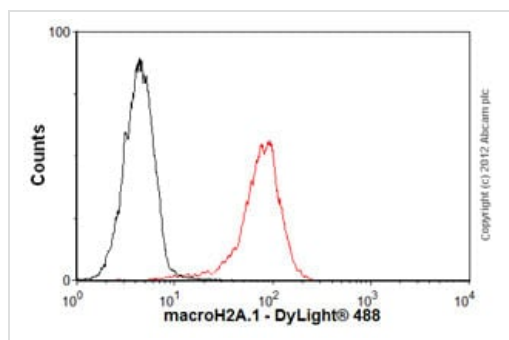
**Lanes 1 - 4:** Merged signal (red and green). Green - ab91528 observed at 40 kDa. Red - loading control, **ab176560**, observed at 50 kDa.

ab91528 was shown to specifically recognize mH2A1 in wild-type HAP1 cells. No band was observed when mH2A1 knockout cells were examined. Wild-type and mH2A1 knockout samples were subjected to SDS-PAGE. ab91528 and **ab176560** (Rabbit anti-alpha Tubulin loading control) were incubated overnight at 4°C at 10 µg/ml and 1/10,000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



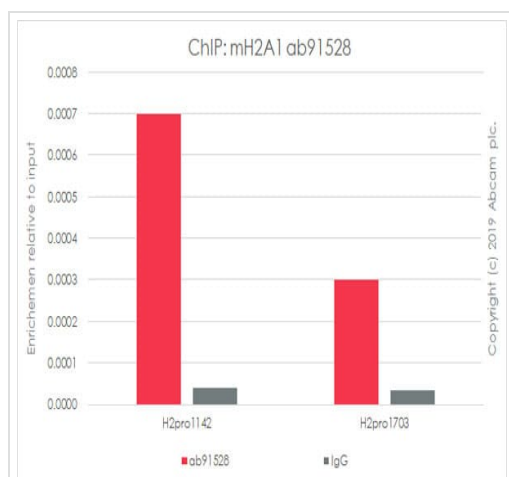
Immunocytochemistry/ Immunofluorescence - Anti-mH2A1 antibody [14G7] (ab91528)

ab91528 staining mH2A1 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 ab91528 at 1 µg/ml and **ab6046**, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with **ab150117**, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and **ab150080**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) 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.



Flow Cytometry (Intracellular) - Anti-mH2A1 antibody [14G7] (ab91528)

Overlay histogram showing HeLa cells stained with ab91528 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab91528, 1µg/1x10<sup>6</sup> cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) ([ab96879](#)) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] ([ab91353](#), 2µg/1x10<sup>6</sup> cells) used under the same conditions. Acquisition of >5,000 events was performed.

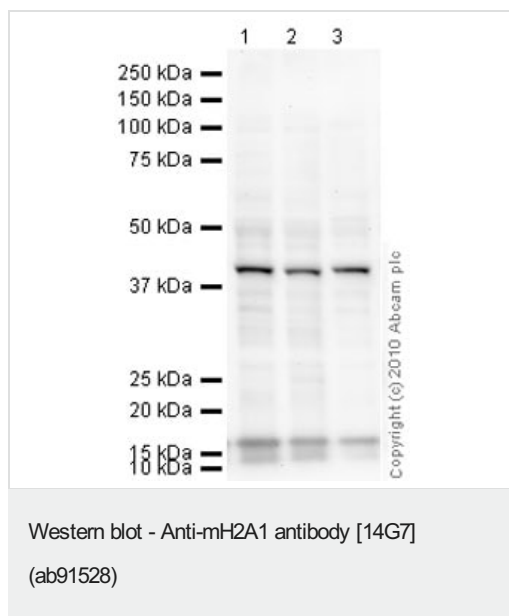


ChIP - Anti-mH2A1 antibody [14G7] (ab91528)

Chromatin was prepared from SK-OV-3 cells according to the Abcam Dual-X-ChIP protocol\*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min. The ChIP was performed with 25 µg of chromatin, 5 µg of ab91528 (red), or 5 µg of mouse normal IgG1 [ab18443](#) (gray) and 25 µl of Protein A/G Dyna beads. 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 commercial primers from Paper PMID: 22589551

\*<http://www.abcam.com/resources?>

keywords=X%20ChIP%20protocol



**All lanes :** Anti-mH2A1 antibody [14G7] (ab91528) at 10 µg/ml

**Lane 1 :** HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

**Lane 2 :** HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate

**Lane 3 :** MCF7 (Human breast adenocarcinoma cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

## Secondary

**All lanes :** Goat polyclonal Secondary Antibody to Mouse IgG - H&L (HRP), pre-adsorbed at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 40 kDa

**Observed band size:** 40 kDa

**Additional bands at:** 14 kDa, 18 kDa. We are unsure as to the identity of these extra bands.

**Exposure time:** 2 minutes

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