abcam

Product datasheet

Anti-Histone H1.2 antibody - ChIP Grade ab4086

KO 評価済

2 Abreviews 21 References 画像数 7

製品の概要

製品名	Anti-Histone H1.2 antibody - ChIP Grade
製品の詳細	Rabbit polyclonal to Histone H1.2 - ChIP Grade
由来種	Rabbit
特異性	This antibody has only been tested on bulk HeLa histones. We expect this antibody to be specific for Histone H1.2 - however we have not tested this specifically on different histone H1 variants.
アプリケーション	**適用あり:** ChIP, WB, IP, ICC/IF, IHC-P
種交差性	**交差種:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab16936**)
ポジティブ・コントロール	HeLa Histone preparation
特記事項	Histone H1.2 is one of five known h1 variants (known as H1.1/2/3/4/5 and/or H1.a/b/c/d/e). The H1 variants differ from each other in the amino acid sequence of their N-terminal regions. For Western Blotting, Abcam recommends blocking in milk in order to increase band clarity. 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

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS

	Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
精製度	Immunogen affinity purified
一次抗体 備考	Histone H1.2 is one of five known h1 variants (known as H1.1/2/3/4/5 and/or H1.a/b/c/d/e). The H1 variants differ from each other in the amino acid sequence of their N-terminal regions.
ポリ/モノ	ポリクローナル
アイソタイプ	IgG

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ab4086の使用に適用されます

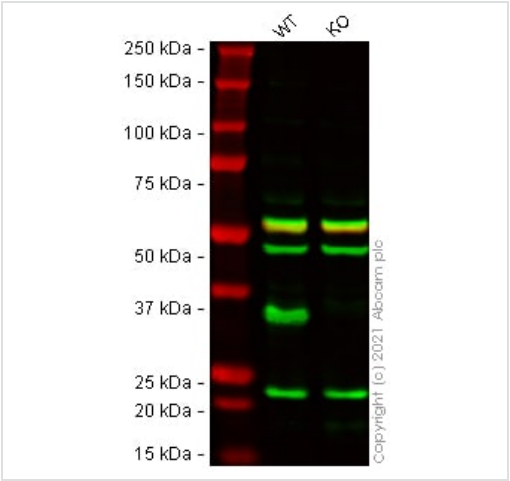
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
ChIP		Use 2-6 µg for 25 µg of chromatin.
WB	★★★★☆ (2)	1/500. Detects a band of approximately 25 kDa (predicted molecular weight: 21 kDa). Blocking in milk is recommended.
IP		Use a concentration of 5 µg/ml.
ICC/IF		Use a concentration of 1 µg/ml.
IHC-P		Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

ターゲット情報

機能	Histones H1 are necessary for the condensation of nucleosome chains into higher order structures.
配列類似性	Belongs to the histone H1/H5 family. Contains 1 H15 (linker histone H1/H5 globular) domain.
細胞内局在	Nucleus. Chromosome.

画像



Western blot - Anti-Histone H1.2 antibody - ChIP Grade (ab4086)

All lanes : Anti-Histone H1.2 antibody - ChIP Grade (ab4086) at 1/500 dilution

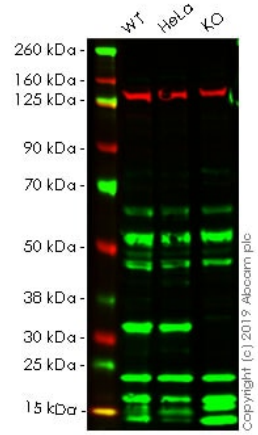
Lane 1 : Wild-type HeLa cell lysate
Lane 2 : HIST1H1C knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 21 kDa
Observed band size: 37 kDa

False colour image of Western blot: Anti-Histone H1.2 antibody - ChIP Grade staining at 1/500 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab4086 was shown to bind specifically to Histone H1.2. A band was observed at 37 kDa in wild-type HeLa cell lysates with no signal observed at this size in HIST1H1C knockout cell line **ab261794** (knockout cell lysate **ab257218**). To generate this image, wild-type and HIST1H1C knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-Histone H1.2 antibody - ChIP Grade (ab4086)

All lanes : Anti-Histone H1.2 antibody - ChIP Grade (ab4086) at 1/500 dilution

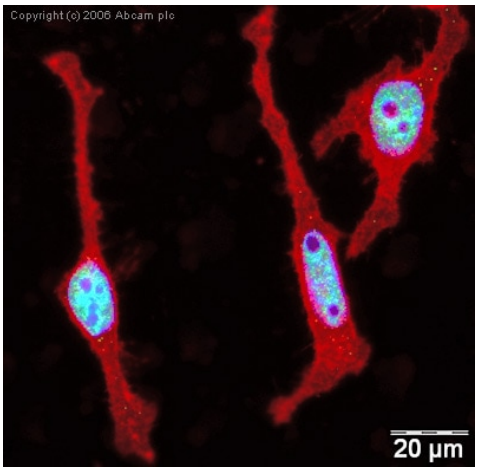
Lane 1 : Wild-type A549 (Human lung carcinoma cell line) whole cell lysate at 20 µg/ml
Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg/ml
Lane 3 : HIST1H1C knockout A549 (Human lung carcinoma cell line) whole cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 21 kDa

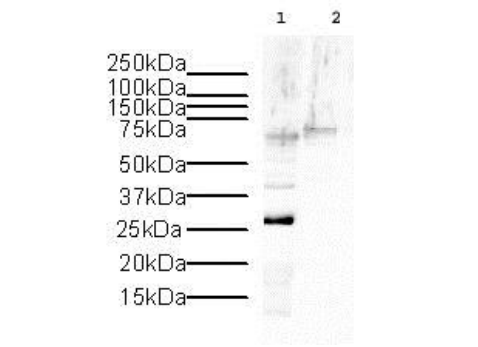
Lanes 1 - 3: Merged signal (red and green). Green - ab4086 observed at 30 kDa. Red - loading control, **ab130007**, observed at 130 kDa.

ab4086 was shown to recognize HIST1H1C in wild-type A549 cells as signal was lost at the expected MW in HeLa knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and HeLa knockout samples were subjected to SDS-PAGE. Ab4086 and **ab130007** (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/500 dilution 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.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H1.2 antibody - ChIP Grade (ab4086)

ICC/IF image of ab4086 stained human HeLa cells. The cells were PFA fixed (3.7% PFA, 10 min) and incubated with the antibody (ab4086, 1µg/ml) for 1h at room temperature. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Image-iT™ FX Signal Enhancer was used as the primary blocking agent, 5% BSA (in TBS-T) was used for all other blocking steps. DAPI was used to stain the cell nuclei (blue). Alexa Fluor® 594 WGA was used to label plasma membranes (red).



Western blot - Anti-Histone H1.2 antibody - ChIP Grade (ab4086)

ab4086 Histone H1.2

The antibody was used at a dilution of 1/500

Lane 1: HeLa Histone (5ug) + ab4086
Lane 2: HeLa Histone (5ug) + ab4086 + 1µg/ml of peptide (Histone H1.2) (**ab16936**)

Secondary ab: Goat polyclonal to Rabbit IgG H&L (HRP) Pre-Adsorbed **ab7090** (1/5000)

Exposure time: 1 minute
Expected molecular weight: 21.3 kDa

ab4086 Histone H1.2

The antibody was used at a dilution of 1/500

Lane 1: HeLa Histone (5ug) + ab4086
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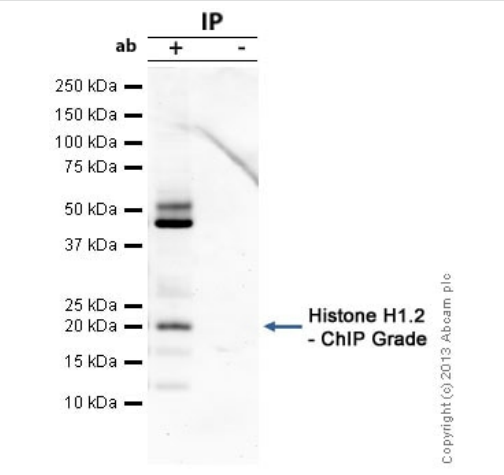
Expected molecular weight: 21.3 kDa



Immunoprecipitation - Anti-Histone H1.2 antibody - ChIP Grade (ab4086)

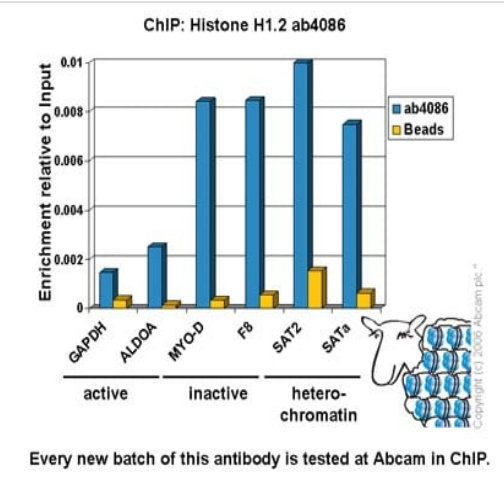
Histone H1.2 - ChIP Grade was immunoprecipitated using 0.5mg HeLa whole cell extract, 5µg of Rabbit polyclonal to and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, HeLa whole cell 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 ab4086.

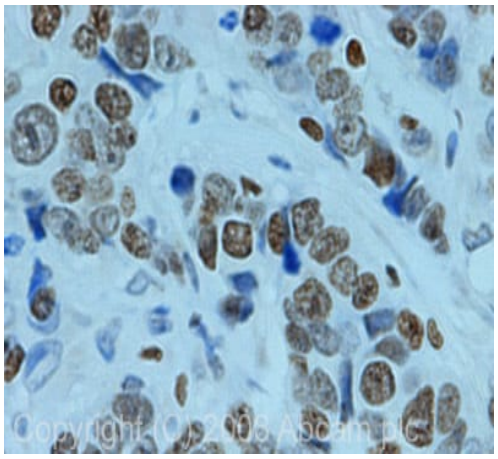
Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (**ab99697**).

Band: 21kDa; Histone H1.2 - ChIP Grade



ChIP - Anti-Histone H1.2 antibody - ChIP Grade (ab4086)

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 25µg of chromatin, 2µg of ab4086 (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 H1.2 antibody - ChIP Grade (ab4086)

IHC image of Histone H1.2 staining in human breast carcinoma FFPE section, performed on a 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 ab4086, 1µg/ml, for 8 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.

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