# abcam

## Product datasheet

## Anti-Histone H2B (acetyl K20) antibody [EPR859] - ChIP Grade ab177430

יובעדער RabMAb

★★★★★ 2 Abreviews 3 References 画像数 11

#### 製品の概要

製品名 Anti-Histone H2B (acetyl K20) antibody [EPR859] - ChIP Grade

製品の詳細 Rabbit monoclonal [EPR859] to Histone H2B (acetyl K20) - ChIP Grade

由来種 Rabbit

アプリケーション 適用あり: Flow Cyt (Intra), PepArr, IHC-P, WB, ICC/IF, ChIP, ChIP-sequencing

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: HeLa and NIH/3T3 whole cell lysate treated with 500 ng/ml Trichostatin A for 4 hours. IHC:

Human and rat colon tissue, Mouse kidney tissue. ICC/IF/Flow Cyt (intra): HeLa cells

特記事項 This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

#### 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 long

term. Avoid freeze / thaw cycle.

バッファー Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

精製度 Protein A purified

ポリモノ モノクローナル

クローン名 **EPR859** 

アイソタイプ ΙgG

**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ab177430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
Flow Cyt (Intra)		1/300. <b>ab172730</b> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
PepArr		Use at an assay dependent concentration.
IHC-P	<b>★★★★ (1)</b>	1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 14 kDa (predicted molecular weight: 14 kDa).
ICC/IF	**** (1)	1/2000.
ChIP		Use 2 µg for 25 µg of chromatin.
ChIP-sequencing		Use at an assay dependent concentration.

#### ターゲット情報

#### 関連性

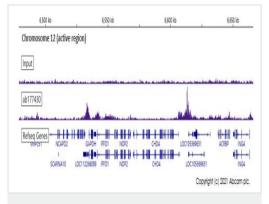
Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility 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 Lvs-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.

#### 細胞内局在

Nuclear

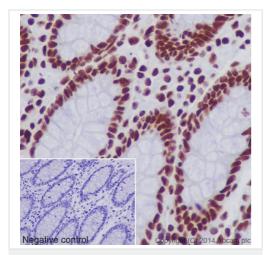
#### 画像



ChIP-sequencing - Anti-Histone H2B (acetyl K20) antibody [EPR859] - ChIP Grade (ab177430)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with  $10^7$  HeLa cells and 4  $\mu$ g of ab177430. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded **here**.

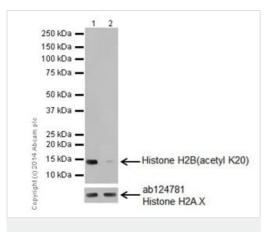


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H2B (acetyl K20) antibody [EPR859] - ChIP Grade (ab177430)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Histone H2B (acetyl K20) with ab177430 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus staining on Human colon is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary antibody.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Histone H2B (acetyl K20) antibody [EPR859] - ChIP Grade (ab177430)

**All lanes :** Anti-Histone H2B (acetyl K20) antibody [EPR859] - ChIP Grade (ab177430) at 1/10000 dilution

Lane 1: HeLa (Human epithelial cells from cervix adenocarcinoma) treated with 500 ng/ml Trichostatin A for 4 hours whole cell lysates

**Lane 2 :** HeLa (Human epithelial cells from cervix adenocarcinoma) untreated whole cell lysates

Lysates/proteins at 10 µg per lane.

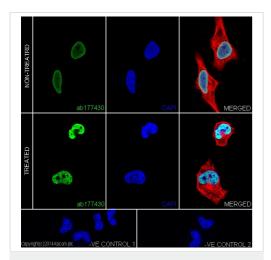
### Secondary

All lanes: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated

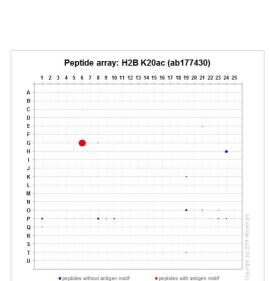
**Predicted band size:** 14 kDa **Observed band size:** 14 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2B (acetyl K20) antibody [EPR859] - ChIP Grade (ab177430)



Peptide Array - Anti-Histone H2B (acetyl K20) antibody [EPR859] - ChIP Grade (ab177430)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells, treated with Trichostatin A (500 ng/ml) for 4 hours or untreated, labeling Histone H2B (acetyl K20) with ab177430 at 1/2000 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/500 dilution (green). Confocal image showing nuclear staining on HeLa cell line is observed. Acetylation level increased after treatment with Trichostatin A (500 ng/ml) for 4 hours. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab7291 (anti-Tubulin mouse mAb) at 1/1000 dilution and ab150120 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

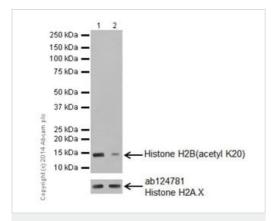
The negative controls are as follows;

1. ab177430 at 1/2000 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
2. <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/1000 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/500 dilution.

ab177430 was tested in Peptide array against 501 different modified and unmodified histone peptides; each peptide is printed on the array at six concentrations (each in triplicate).

Circle area represents affinity between the antibody and a peptide: all antigen-containing peptides are displayed as red circles, all other peptides as blue circles. The affinity is calculated as area under curve when antibody binding values are plotted against the corresponding peptide concentration. Each circle area is normalized to the peptide with the strongest affinity.

The complete dataset, including full list of all peptides and information on the position of each peptide in the diagram, can be downloaded **here**.



Western blot - Anti-Histone H2B (acetyl K20) antibody [EPR859] - ChIP Grade (ab177430)

**All lanes :** Anti-Histone H2B (acetyl K20) antibody [EPR859] - ChIP Grade (ab177430) at 1/1000 dilution

**Lane 1**: NIH/3T3 (Mouse embyro fibroblast cells) treated with 500 ng/ml Trichostatin A for 4 hours whole cell lysates 10ug

Lane 2: NIH/3T3 (Mouse embyro fibroblast cells) untreated whole cell lysates 10ug

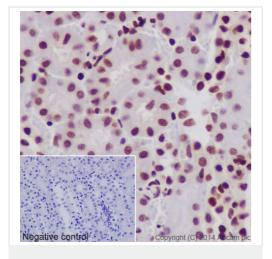
#### **Secondary**

**All lanes :** Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 14 kDa **Observed band size:** 14 kDa

Exposure time: 10 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

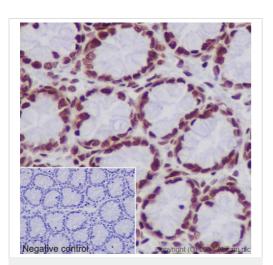


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H2B (acetyl K20) antibody [EPR859] - ChIP Grade (ab177430)

Immunohistochemical analysis of paraffin-embedded Mouse kidney tissue labeling Histone H2B (acetyl K20) with ab177430 at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus staining on mouse kidney is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary antibody.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

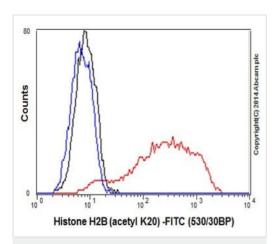


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H2B (acetyl K20) antibody [EPR859] - ChIP Grade (ab177430)

Immunohistochemical analysis of paraffin-embedded Rat colon tissue labeling Histone H2B (acetyl K20) with ab177430 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nucleus staining on Rat colon is observed. Counter stained with Hematoxylin.

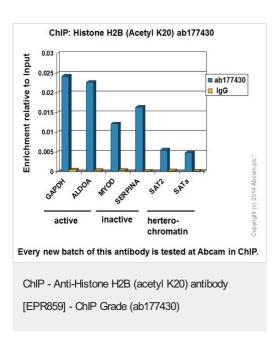
Negative control: Using PBS instead of primary antibody.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

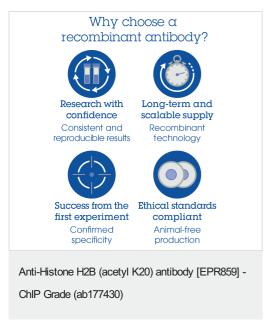


Flow Cytometry (Intracellular) - Anti-Histone H2B (acetyl K20) antibody [EPR859] - ChIP Grade (ab177430)

Intracellular flow cytometric analysis of 2% paraformaldehyde-fixed HeLa (Human epithelial cells from cervix adenocarcinoma) cells treated with 500ng/ml Trichostatin A for 4 hours, labeling Histone H2B (acetyl K20) with ab177430 at 1/300 dilution (red) compared with a rabbit monoclonal IgG isotype control (black) and a unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (FITC) at 1/150 dilution was used as the secondary antibody.



Chromatin was prepared from HeLa (Human epithelial cells from cervix adenocarcinoma) 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 ab177430 (blue), and 20µl of Anti rabbit lgG sepharose beads. 2µg of rabbit normal lgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach). Primers and probes are located in the first kb of the transcribed region.



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