# abcam

# Product datasheet

# Anti-Histone H2B (acetyl K16) antibody [EPR17598] - ChIP Grade ab177427



★★★★★ 2 Abreviews 1 References 9 Images

#### Overview

Product name Anti-Histone H2B (acetyl K16) antibody [EPR17598] - ChIP Grade

**Description** Rabbit monoclonal [EPR17598] to Histone H2B (acetyl K16) - ChIP Grade

Host species Rabbit

Tested applications Suitable for: PepArr, IHC-P, WB, ICC/IF, ChIP-sequencing

**Species reactivity** Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa and NIH/3T3 treated with 500 ng/ml Trichostatin A for 4 hours whole cell lysates. IHC-P:

Human colon, mouse liver and rat pancreas tissues. ICC/IF: HeLa cells.

General notes 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<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

# **Properties**

Form Liquid

Storage instructions 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.

**Storage buffer** Preservative: 0.01% Sodium azide

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

Purity Protein A purified

ClonalityMonoclonalClone numberEPR17598

**Isotype** IgG

1

#### **Applications**

#### The Abpromise quarantee

Our Abpromise quarantee covers the use of ab177427 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
PepArr		Use at an assay dependent concentration.
IHC-P	**** (1)	1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/2000. Detects a band of approximately 14 kDa (predicted molecular weight: 14 kDa).
ICC/IF	**** <u>(1)</u>	1/2000.
ChIP-sequencing		Use at an assay dependent concentration.

# **Target**

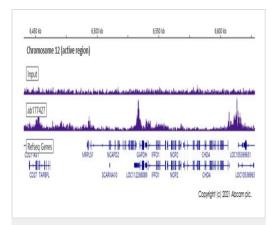
#### Relevance

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.

**Cellular localization** 

Nuclear

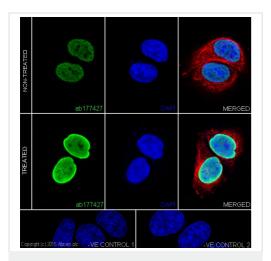
## **Images**



ChIP-sequencing - Anti-Histone H2B (acetyl K16) antibody [EPR17598] - ChIP Grade (ab177427)

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 ab177427. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

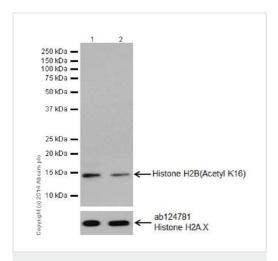
Additional screenshots of mapped reads can be downloaded **here**.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2B (acetyl K16) antibody [EPR17598] -ChIP Grade (ab177427) Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Histone H2B (acetyl K16) with ab177427 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. 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:

-ve control 1: ab177427 at 1/2000 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution. -ve control 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.



Western blot - Anti-Histone H2B (acetyl K16) antibody [EPR17598] - ChIP Grade (ab177427)

All lanes: Anti-Histone H2B (acetyl K16) antibody [EPR17598] -ChIP Grade (ab177427) at 1/50000 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: Untreated HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysates

Lysates/proteins at 10 µg per lane.

# Secondary

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

Predicted band size: 14 kDa

Observed band size: 14 kDa

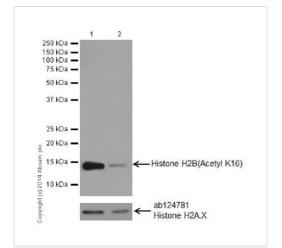
Blocking/Dilution buffer: 5% NFDM/TBST.

All lanes: Anti-Histone H2B (acetyl K16) antibody [EPR17598] -ChIP Grade (ab177427) at 1/2000 dilution

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

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

Lysates/proteins at 10 µg per lane.



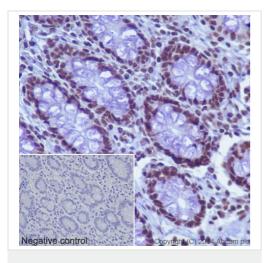
Western blot - Anti-Histone H2B (acetyl K16) antibody [EPR17598] - ChIP Grade (ab177427)

## **Secondary**

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

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

Blocking/Dilution buffer: 5% NFDM/TBST.

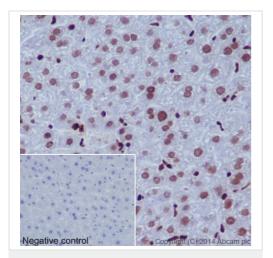


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H2B (acetyl K16) antibody [EPR17598] - ChIP Grade (ab177427)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Histone H2B (acetyl K16) with ab177427 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nucleus staining on glandular epithelium of Human colon tissue is observed. Counter stained with Hematoxylin.

Negative control: Uses PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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

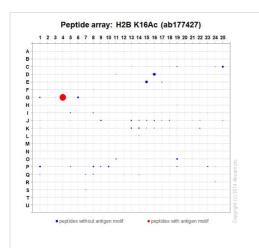


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H2B (acetyl K16) antibody [EPR17598] - ChIP Grade (ab177427)

Immunohistochemical analysis of paraffin-embedded mouse liver tissue labeling Histone H2B (acetyl K16) with ab177427 at 1/2000 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nucleus staining on mouse liver tissue is observed. Counter stained with Hematoxylin.

Negative control: Uses PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution.

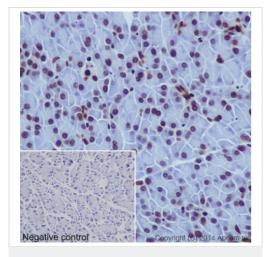
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Peptide Array - Anti-Histone H2B (acetyl K16) antibody [EPR17598] - ChIP Grade (ab177427) ab177427 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.

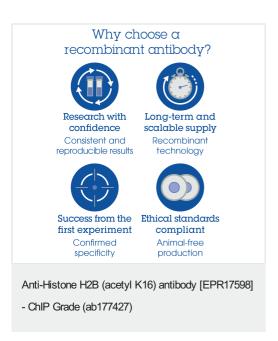


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H2B (acetyl K16) antibody [EPR17598] - ChIP Grade (ab177427)

Immunohistochemical analysis of paraffin-embedded rat pancreas tissue labeling Histone H2B (acetyl K16) with ab177427 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Nucleus staining on glandular epithelium of rat pancreas tissue is observed. Counter stained with Hematoxylin.

Negative control: Uses PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

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



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

# Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <a href="https://www.abcam.com/abpromise">https://www.abcam.com/abpromise</a> or contact our technical team.

#### Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors