abcam

Product datasheet

Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade ab40886

Recombinant RabMAb

4 Abreviews 14 References 13 Images

Overview

Product name	Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade
Description	Rabbit monoclonal [EP857Y] to Histone H2B (acetyl K5) - ChIP Grade
Host species	Rabbit
Specificity	There is cross-reactivity with H3K27Ac (Histone H3 acetylated on Lys 27). The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
Tested applications	**Suitable for:** WB, ChIP-sequencing, ChIP, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Caenorhabditis elegans, Drosophila melanogaster
Immunogen	Synthetic peptide within Human Histone H2B aa 1-100 (acetyl K5). The exact sequence is proprietary. (Peptide available as **ab203469**)
Positive control	WB: HeLa (500ng/ml trichostatin A for 4 hours), NIH/3T3 (500ng/ml trichostatin A for 4 hours) and C6 (500ng/ml trichostatin A for 4 hours) cell lysates. IHC-P: Human hepatocellular carcinoma and urinary bladder carcinoma. ICC/IF: HeLa and A431 cells. ChIP: HeLa cells.
General notes	We do not guarantee IHC-P for mouse and rat. 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**.

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	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP857Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab40886 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
WB		1/10000. Detects a band of approximately 14 kDa (predicted molecular weight: 14 kDa). **For unpurified use at 1/50000.**
ChIP-sequencing		Use 4µg for 10^7 cells.
ChIP	★★★★☆ **(1)**	Use 2 µg for 25 µg of chromatin.
IHC-P		1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
ICC/IF		1/1000. **For unpurified use at 1/250 - 1/500.**

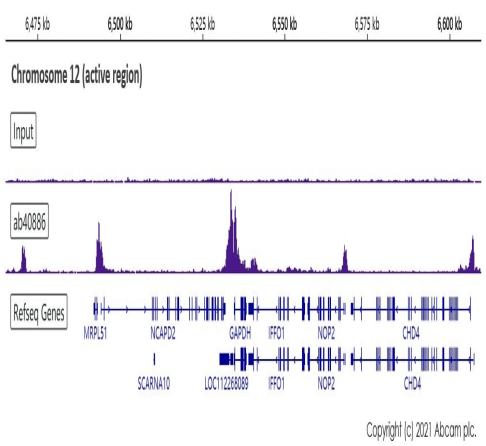
Target

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

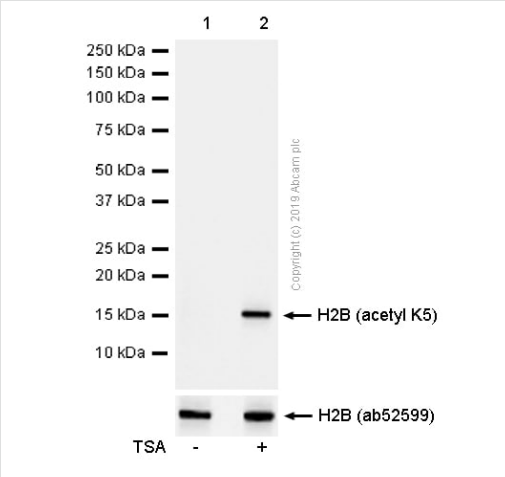
Images



ChIP-sequencing - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

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 µg of ab40886 [EP857Y]. 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**.



Western blot - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

All lanes : Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886) at 1/10000 dilution (Purified)

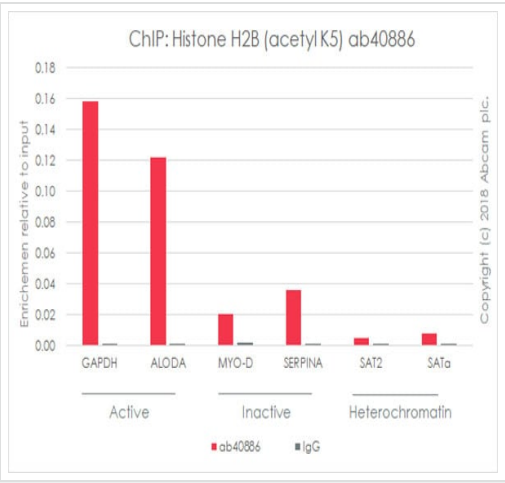
Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) treated with 500ng/ml trichostatin A for 4 hours whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

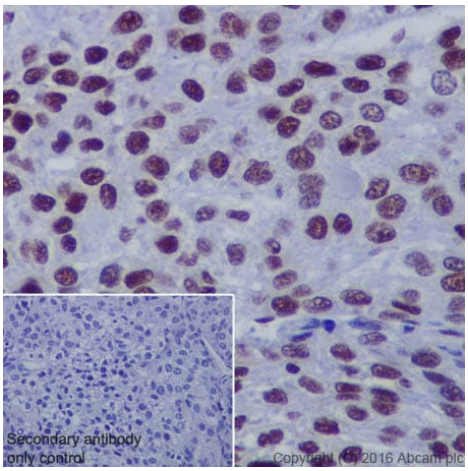
Predicted band size: 14 kDa
Observed band size: 14 kDa

Blocking/Diluting Buffer and concentration: 5% NFDM/TBST



ChIP - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

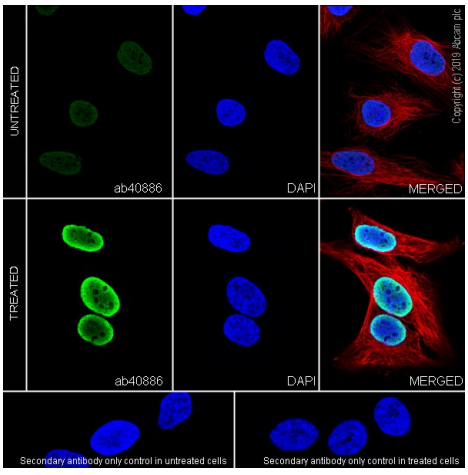
Chromatin was prepared from HeLa (Human cervix adenocarcinoma epithelial cell) cells according to the Abcam X-ChIP protocol*. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25 µg of chromatin, 2µg of ab40886 (red), or 2 µg of rabbit normal IgG (gray) and 20 µl of Protein A/G sepharose 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 located in the first kb of the transcribed region.
*http://www.abcam.com/resources?keywords=X%20ChIP%20protocol



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human hepatocellular carcinoma tissue sections labeling Histone H2B with purified ab40886 at 1/100 dilution (5.42 µg/mL). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody.

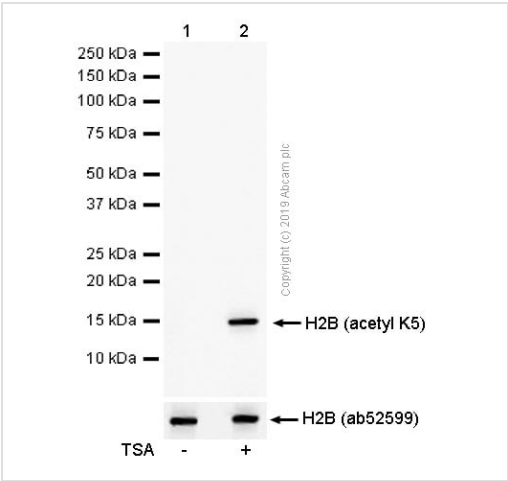
Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) treated with 500 ng/mL TSA for 4 hours cells labeling Histone H2B with purified ab40886 at 1/1000 dilution (0.54 µg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain.

PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

All lanes : Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886) at 1/10000 dilution (Purified)

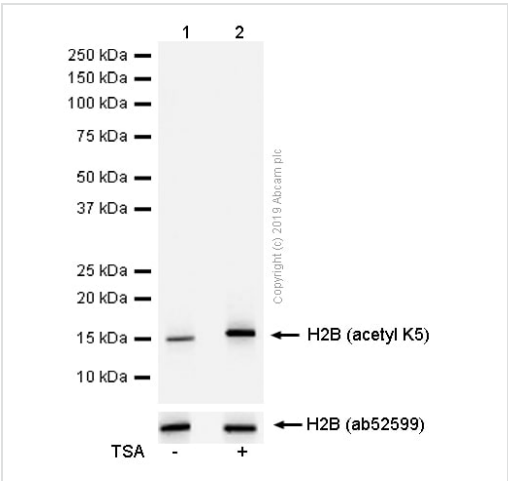
Lane 1 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) treated with 500ng/ml trichostatin A for 4 hours whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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

Blocking/Diluting Buffer and concentration: 5% NFDM/TBST



Western blot - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

All lanes : Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886) at 1/10000 dilution (Purified)

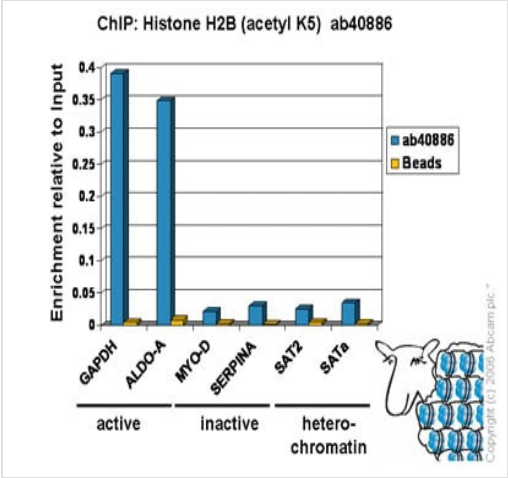
Lane 1 : C6 (Rat glial tumor glial cell) whole cell lysates
Lane 2 : C6 (Rat glial tumor glial cell) treated with 500ng/ml trichostatin A for 4 hours whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

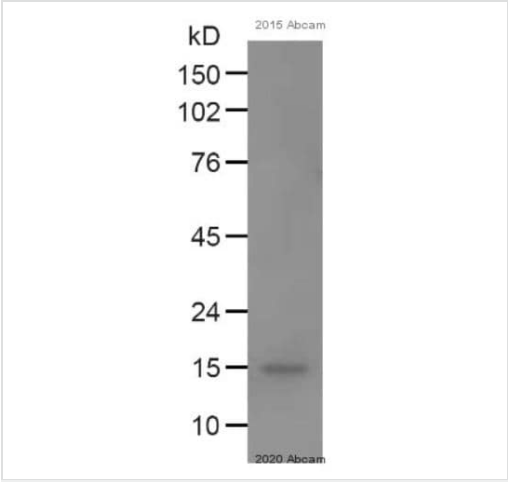
Predicted band size: 14 kDa
Observed band size: 14 kDa

Blocking/Diluting Buffer and concentration: 5% NFDM/TBST



ChIP - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

Chromatin was prepared from HeLa (Human cervix adenocarcinoma epithelial cell) cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25µg of chromatin, 6µl of unpurified ab40886 (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.



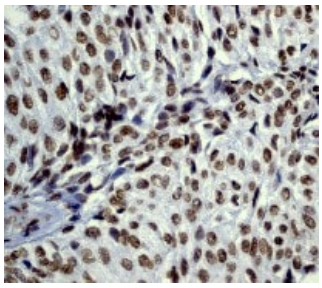
Western blot - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886) at 1/3000 dilution + Drosophila melanogaster lysate (Fruit fly larvae) at 20 µg

Secondary

Anti-rabbit IgG HRP at 1/6000 dilution

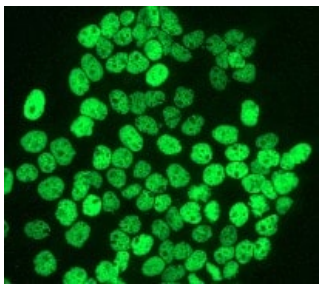
Predicted band size: 14 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

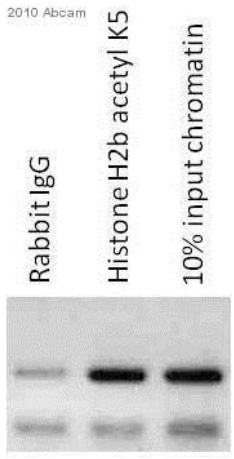
ab40886 (unpurified) staining human bladder carcinoma for Histone H2B expression (1/250 dilution)

Heat mediated antigen retrieval was performed with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

ab40886 (unpurified) (1/250) staining A431 (Human epidermoid carcinoma cell line) cells by immunofluorescence.



ChIP - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

This image is courtesy of an anonymous abreview.

ab40886 (unpurified) at a 1/600 dilution for ChIP analysis of mouse dorsal skin epidermis whole tissue lysate, incubated for 15 hours at 4°C with ChIP dilution buffer. Cross-linking (X-ChIP) using 1% formaldehyde for 10 minutes.Detection step: Semiquantitative PCR.Negative control: Rabbit IgG.Cells treated with active vitamin D3.

Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

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 **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

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