abcam

Product datasheet

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

ייבערעדער RabMAb

★★★★ <u>4 Abreviews</u> <u>1</u>4 References 画像数 13

製品の概要

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

製品の詳細 Rabbit monoclonal [EP857Y] to Histone H2B (acetyl K5) - ChIP Grade

由来種 Rabbit

特異性 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.

アプリケーション 適用あり: WB, ChIP-sequencing, ChIP, IHC-P, ICC/IF

種交差性 交差種: Mouse. Rat. Human

交差が予測される動物種: Caenorhabditis elegans, Drosophila melanogaster

免疫原 Synthetic peptide within Human Histone H2B aa 1-100 (acetyl K5). The exact sequence is

(Peptide available as ab203469)

ポジティブ・コントロール 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.

We do not guarantee IHC-P for mouse and rat. 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 pH: 7.2

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 EP857Y

アイソタイプ lgG

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ab40886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
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.

ターゲット情報

関連性

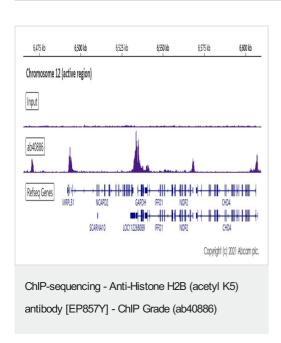
Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility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

細胞内局在

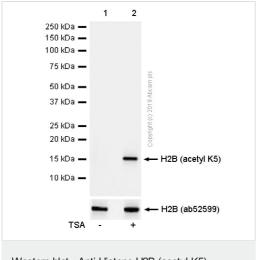
Nuclear

画像



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

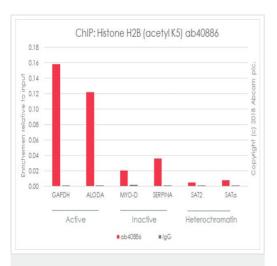
Lysates/proteins at 15 µg per lane.

Secondary

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

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

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

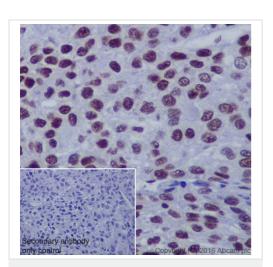


ChIP - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

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, $2\mu g$ 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.

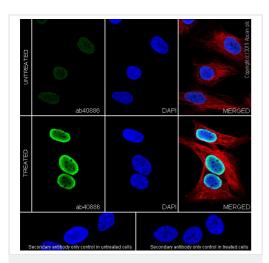
*http://www.abcam.com/resources? keywords=X%20ChIP%20protocol



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

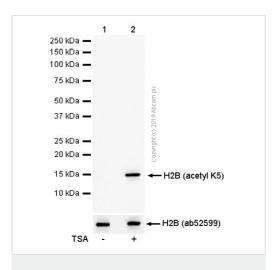
Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



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 μ g/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with <u>ab195889</u> Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) 1/200 (2.5 μ g/mL). Goat anti rabbit lgG (Alexa Fluor[®] 488, <u>ab150077</u>) was used as the secondary antibody at 1/1000 (2 μ g/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 lysatesLane 2: NIH/3T3 (Mouse embryonic fibroblast) treated with500ng/ml trichostatin A for 4 hours whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

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

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

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.

250 kDa — 150 kDa — 100 kDa — 75 kDa — 37 kDa — 37 kDa — 25 kDa — 20 kDa — 15 kDa — 4 H2B (acetyl K5) TSA - +

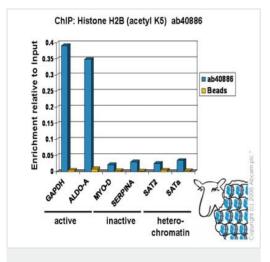
Western blot - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

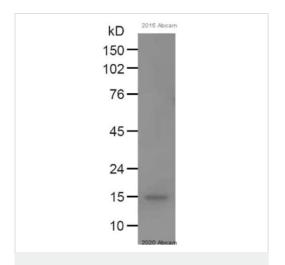
Predicted band size: 14 kDa **Observed band size:** 14 kDa

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.



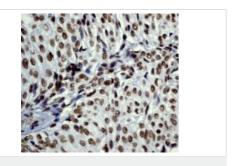
Western blot - Anti-Histone H2B (acetyl K5) antibody [EP857Y] - ChIP Grade (ab40886)

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

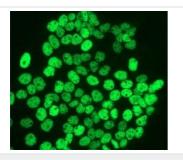
Predicted band size: 14 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

Rabbit IgG
Histone H2b acetyl K5
10% input chromatin

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

This image is courtesy of an anonymous abreview.

Why choose a

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.



Success from the first experiment Confirmed specificity Ethical standards compliant Animal-free production

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

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