abcam

Product datasheet

Anti-Histone H2B (crotonyl K12) antibody [EPR17593] - BSA and Azide free ab203950



RabMAb

7 Images

Overview

Product name Anti-Histone H2B (crotonyl K12) antibody [EPR17593] - BSA and Azide free

Description Rabbit monoclonal [EPR17593] to Histone H2B (crotonyl K12) - BSA and Azide free

Host species Rabbit

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

Species reactivity Reacts with: Mouse, Rat, Human

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

General notes ab203950 is the carrier-free version of <u>ab177420</u>.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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

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Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR17593

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab203950 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		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 14 kDa (predicted molecular weight: 14 kDa).
ICC/IF		Use at an assay dependent concentration.

Target

Relevance

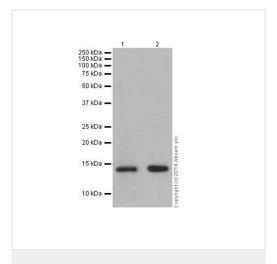
Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility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



Western blot - Anti-Histone H2B (crotonyl K12) antibody [EPR17593] - BSA and Azide free (ab203950) **All lanes :** Anti-Histone H2B (crotonyl K12) antibody [EPR17593] (**ab177420**) at 1/1000 dilution

Lane 1 : NIH/3T3 whole cell lysates

Lane 2 : HeLa whole cell lysates

Lysates/proteins at 10 µg per lane.

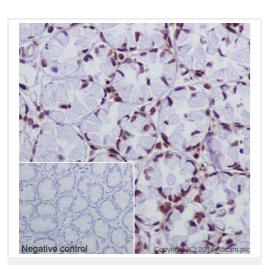
Secondary

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

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

This data was developed using <u>ab177420</u>, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.

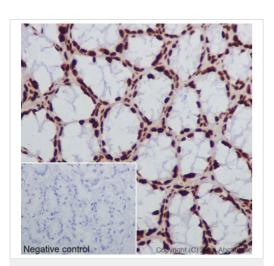


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H2B (crotonyl K12) antibody [EPR17593] - BSA and Azide free (ab203950)

This data was developed using **ab177420**, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling Histone H2B (crotonyl K12) with <u>ab177420</u> at 1/1000 dilution followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) secondary antibody at 1/500 dilution. Nucleus staining on glandular epithelium of rat colon tissue is observed. Counter stained with Hematoxylin.

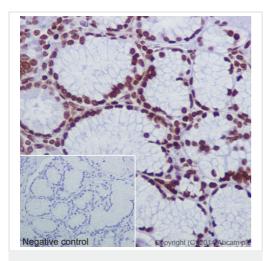
Negative control: Using PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H2B (crotonyl K12) antibody [EPR17593] - BSA and Azide free (ab203950)

This data was developed using <u>ab177420</u>, the same antibody clone in a different buffer formulation.Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling Histone H2B (crotonyl K12) with <u>ab177420</u> at 1/1000 dilution followed by Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) secondary antibody at 1/500 dilution. Nucleus staining on glandular epithelium of mouse colon tissue is observed. Counter stained with Hematoxylin.

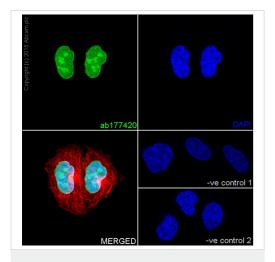
Negative control: Using PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) secondary antibody at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H2B (crotonyl K12) antibody [EPR17593] - BSA and Azide free (ab203950)

This data was developed using <u>ab177420</u>, the same antibody clone in a different buffer formulation.Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Histone H2B (crotonyl K12) with <u>ab177420</u> at 1/1000 dilution followed by Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) secondary antibody at 1/500 dilution. Nucleus staining on glandular epithelium of Human colon tissue is observed. Counter stained with Hematoxylin.

Negative control: Using PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) secondary antibody at 1/500 dilution. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

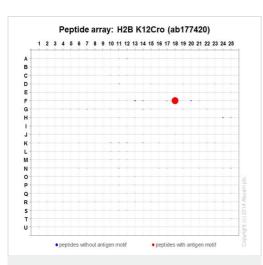


Immunocytochemistry/ Immunofluorescence - Anti-Histone H2B (crotonyl K12) antibody [EPR17593] -BSA and Azide free (ab203950)

This data was developed using <u>ab177420</u>, the same antibody clone in a different buffer formulation.Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells labeling Histone H2B (crotonyl K12) with <u>ab177420</u> at 1/100 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (<u>ab150077</u>) secondary antibody at 1/400 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/500 dilution and <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:

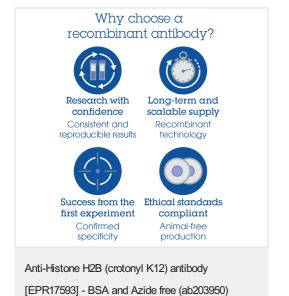
-ve control 1: <u>ab177420</u> at 1/100 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/500 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/400 dilution.



Peptide Array - Anti-Histone H2B (crotonyl K12) antibody [EPR17593] - BSA and Azide free (ab203950) This data was developed using <u>ab177420</u>, the same antibody clone in a different buffer formulation.<u>ab177420</u> 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**.



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