abcam

Product datasheet

Anti-Histone H4 antibody [EPR16599] - BSA and Azide free ab232371



7 Images

Overview

Immunogen

Product name Anti-Histone H4 antibody [EPR16599] - BSA and Azide free

Description Rabbit monoclonal [EPR16599] to Histone H4 - BSA and Azide free

Host species Rabbit

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

Species reactivity Reacts with: Mouse, Rat, Human, Drosophila melanogaster, Recombinant fragment

Predicted to work with: a wide range of other species

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

Positive control IHC-P: Human colon tissue.

General notes ab232371 is the carrier-free version of ab177840.

> Our carrier-free 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 cellbased 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**.

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Properties

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 EPR16599

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab232371 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
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.
ChIP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 11 kDa (predicted molecular weight: 11 kDa). We recommend using 3% milk as the blocking agent for Western blot.
ICC/IF		Use at an assay dependent concentration.
PepArr		Use at an assay dependent concentration.

Target

Function 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.

Sequence similarities Belongs to the histone H4 family.

Post-translational modifications

 $Acetylation\ at\ Lys-6\ (H4K5ac),\ Lys-9\ (H4K8ac),\ Lys-13\ (H4K12ac)\ and\ Lys-17\ (H4K16ac)\ occurs$

in coding regions of the genome but not in heterochromatin.

Citrullination at Arg-4 (H4R3ci) by PADI4 impairs methylation.

Monomethylation and asymmetric dimethylation at Arg-4 (H4R3me1 and H4R3me2a, respectively) by PRMT1 favors acetylation at Lys-9 (H4K8ac) and Lys-13 (H4K12ac). Demethylation is performed by JMJD6. Symmetric dimethylation on Arg-4 (H4R3me2s) by the PRDM1/PRMT5 complex may play a crucial role in the germ-cell lineage.

Monomethylated, dimethylated or trimethylated at Lys-21 (H4K20me1, H4K20me2, H4K20me3). Monomethylation is performed by SET8. Trimethylation is performed by SUV420H1 and SUV420H2 and induces gene silencing.

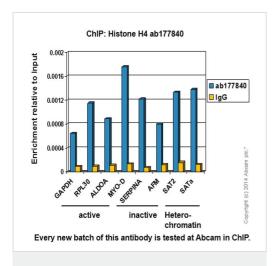
Ubiquitinated by the CUL4-DDB-RBX1 complex in response to ultraviolet irradiation. This may weaken the interaction between histones and DNA and facilitate DNA accessibility to repair proteins. Monoubiquitinated at Lys-92 of histone H4 (H4K91ub1) in response to DNA damage. The exact role of H4K91ub1 in DNA damage response is still unclear but it may function as a licensing signal for additional histone H4 post-translational modifications such as H4 Lys-21 methylation (H4K20me).

Sumoylated, which is associated with transcriptional repression.

Cellular localization

Nucleus. Chromosome.

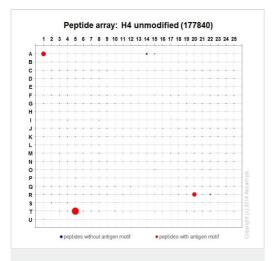
Images



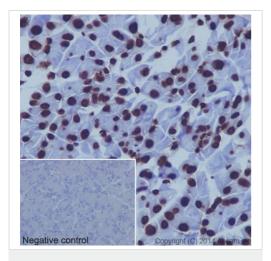
ChIP - Anti-Histone H4 antibody [EPR16599] - BSA and Azide free (ab232371)

Chromatin was prepared from Hela cells according to the Abcam X-ChIP protocol. Cells were fixed with 0.75% formaldehyde for 10min. The ChIP was performed with 25µg of chromatin, 2µg of ab177840 (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 approach). Primers are located in the first kb of the transcribed region.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab177840</u>).



Peptide Array - Anti-Histone H4 antibody [EPR16599] - BSA and Azide free (ab232371)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H4 antibody
[EPR16599] - BSA and Azide free (ab232371)

<u>ab177840</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**.

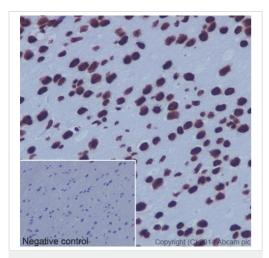
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab177840</u>).

Immunohistochemical analysis of paraffin-embedded Mouse pancreas tissue labeling Histone H4 with **ab177840** at 1/2000 dilution, followed by prediluted Goat Anti-Rabbit lgG H&L (HRP). Nucleus staining on glandular epithelium of mouse pancreas tissue is observed. Counter stained with Hematoxylin.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab177840).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



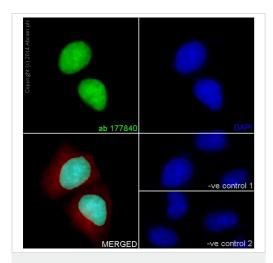
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H4 antibody
[EPR16599] - BSA and Azide free (ab232371)

Immunohistochemical analysis of paraffin-embedded Rat cerebral cortex tissue labeling Histone H4 with <u>ab177840</u> at 1/2000 dilution, followed by prediluted Goat Anti-Rabbit IgG H&L (HRP). Nuclear staining on neuron cells of cerebral cortex tissue is observed. Counter stained with Hematoxylin.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab177840</u>).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 antibody [EPR16599] - BSA and Azide free (ab232371)

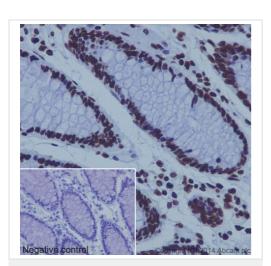
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells labeling Histone H4 with **ab177840** at 1/100 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/400 dilution (green). Nuclear staining on HeLa cell line is observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:

ab177840 at 1/100 dilution followed by ab150120
 (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.

 ab7291 (anti-Tubulin mouse mAb) at 1/500 dilution followed by ab150077 (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/400 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab177840).



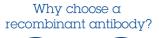
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H4 antibody [EPR16599] - BSA and Azide free (ab232371)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Histone H4 with ab177840 at 1/2000 dilution, followed by prediluted Goat Anti-Rabbit IgG H&L (HRP). Nucleus staining on glandular epithelium of Human colon tissue is observed. Counter stained with Hematoxylin.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab177840).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.





Long-term and scalable supply Recombinant technology





first experiment Confirmed specificity

production

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