abcam

Product datasheet

Anti-Histone H4 (acetyl K16) antibody [EPR1004] ab109463

Recombinant RabMAb

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Overview

Product name	Anti-Histone H4 (acetyl K16) antibody [EPR1004]
Description	Rabbit monoclonal [EPR1004] to Histone H4 (acetyl K16)
Host species	Rabbit
Specificity	This antibody only detects Histone H4 acetylated on Lysine 16.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, ChIC/CUT&RUN-seq **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, C6 and mouse spleen cell lysates - treated with TSA. IHC-P: Human testis, transitional cell carcinoma and colon tissues. ICC/IF: HeLa cells treated with TSA. Flow Cyt (intra): HeLa cells. ChIC/CUT&RUN-Seq: 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® 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. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 59% PBS, 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR1004
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab109463 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
Flow Cyt (Intra)		1/100 - 1/200. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (4)	1/1000 - 1/2000. Detects a band of approximately 11 kDa (predicted molecular weight: 11 kDa).
IHC-P	★★★★★ (2)	1/100 - 1/200. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF	★★★★☆ (1)	1/100 - 1/200.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 2 µg

Application notes Is unsuitable for IP.

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.

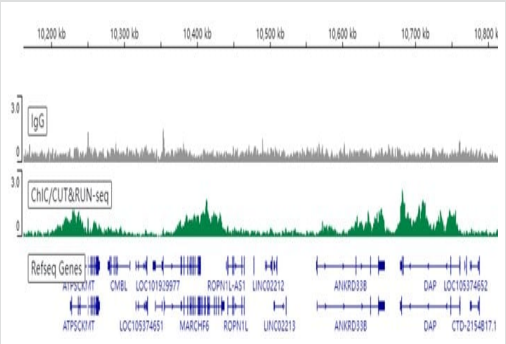
Sequence similarities Belongs to the histone H4 family.

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

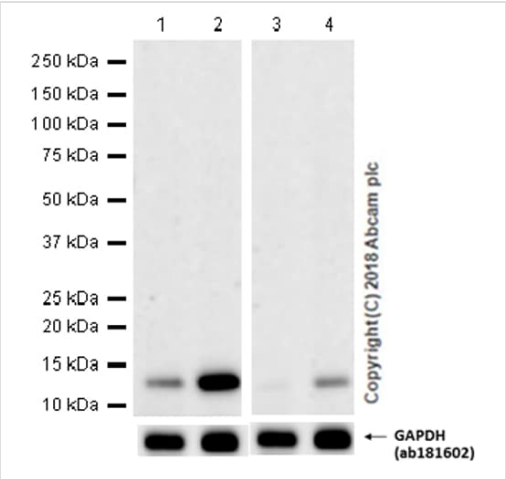


ChIC/CUT&RUN sequencing - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and 2 µg of ab109463 [EPR1004]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown.

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

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Western blot - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Lanes 1-2 : Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463) at 1/6000 dilution
Lanes 3-4 : Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463) at 1/24000 dilution

Lanes 1 & 3 : Untreated C6 (Rat glial tumor glial cell) whole cell lysate
Lanes 2 & 4 : C6 (Rat glial tumor glial cell) treated with Trichostatin A (final concentration is 500ng/ml) for 4 hours whole cell lysate

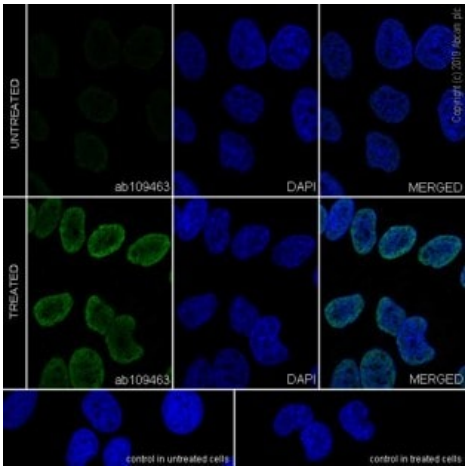
Lysates/proteins at 20 µg per lane.

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

Predicted band size: 11 kDa

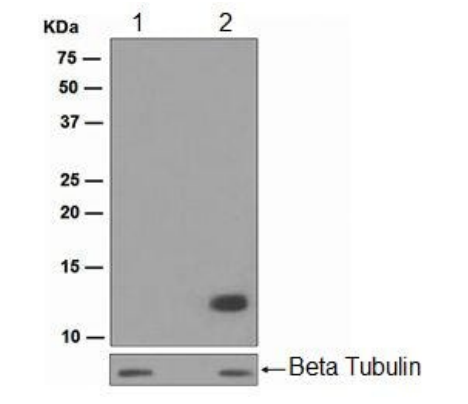
Exposure time: 3 minutes

Blocking/Diluting buffer and concentration 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Immunocytochemistry/ Immunofluorescence analysis of untreated HeLa cells (top row) and HeLa+ TSA(500ng/ml, 4h) cells (middle row) labeling Histone H4 (acetyl K16) with ab109463 at 1/500. Goat anti rabbit IgG(Alexa Fluor® 488); **ab150077** at 1/1000 dilution was used as the secondary antibody. Cells were fixed with 4% paraformaldehyde and permeabilised with 0.1% tritonX-100. DAPI (blue) was used as a nuclear counterstain.



Western blot - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

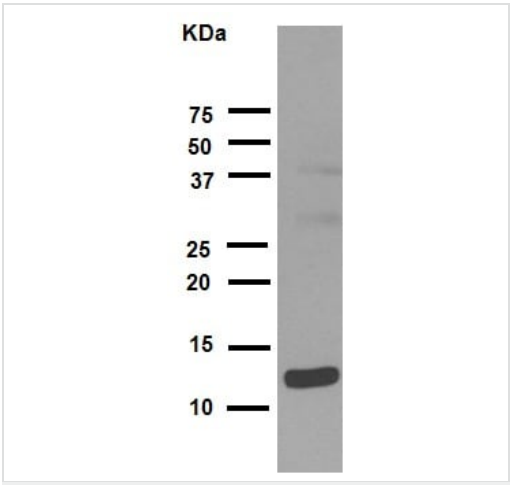
All lanes : Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463) at 1/1000 dilution (unpurified)

Lane 1 : HeLa cell lysates, untreated
Lane 2 : HeLa cell lysates treated with TSA

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP-labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 11 kDa
Observed band size: 11 kDa



Western blot - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463) at 1/1000 dilution (unpurified) + HeLa cell lysate - treated with TSA at 10 µg

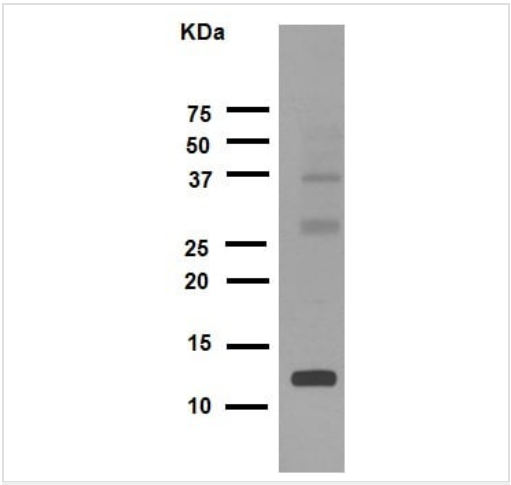
Secondary

Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 11 kDa
Observed band size: 11 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463) at 1/1500 dilution (purified) + HeLa cell lysate - treated with TSA at 10 µg

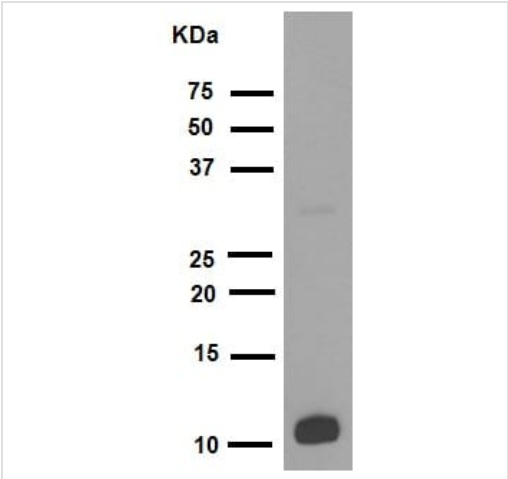
Secondary

Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 11 kDa
Observed band size: 11 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463) at 1/1000 dilution (unpurified) + C6 cell lysate - treated with TSA at 10 µg

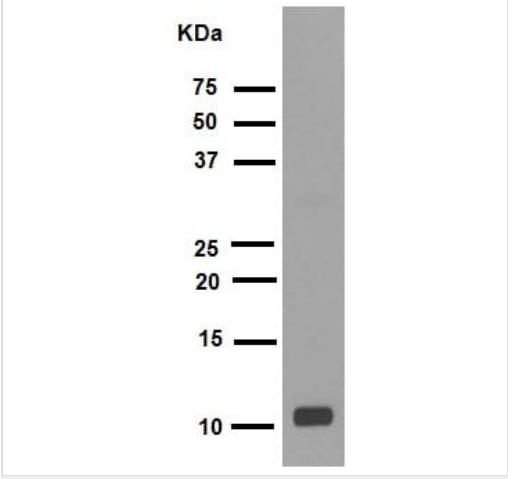
Secondary

Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 11 kDa
Observed band size: 11 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463) at 1/1500 dilution (unpurified) + C6 cell lysate - treated with TSA at 10 µg

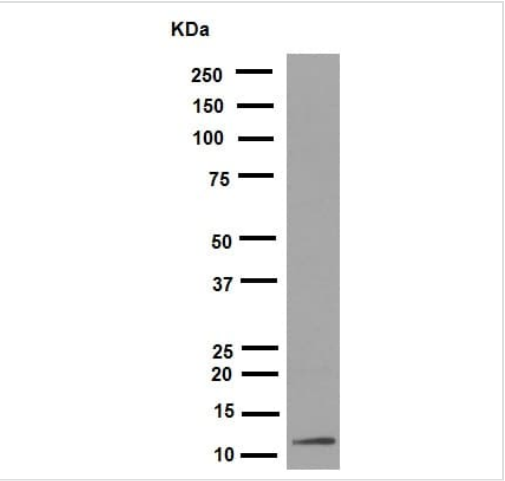
Secondary

Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 11 kDa
Observed band size: 11 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463) at 1/1000 dilution (purified) + Mouse spleen tissue lysate at 10 µg

Secondary

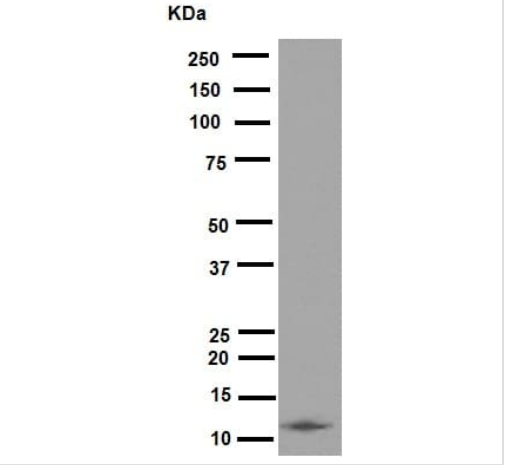
Peroxidase-conjugated goat anti-rabbit IgG (H+L)

Predicted band size: 11 kDa

Observed band size: 11 kDa

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463) at 1/1500 dilution (purified) + Mouse spleen tissue lysate at 10 µg

Secondary

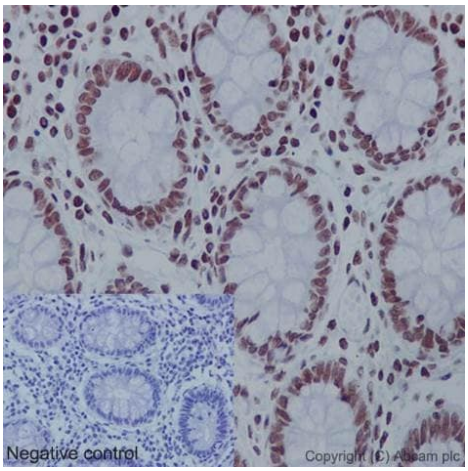
Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 11 kDa

Observed band size: 11 kDa

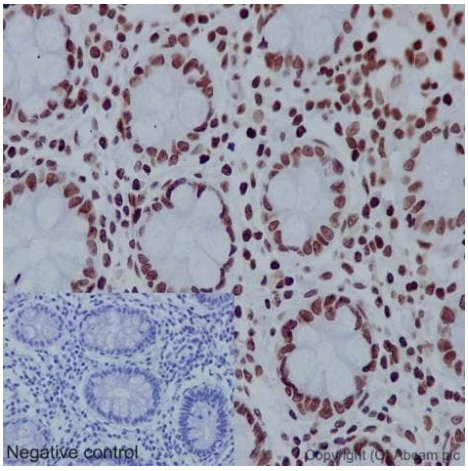
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



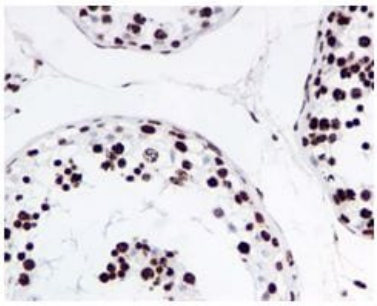
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon tissue labelling Histone H4 (acetyl K16) with unpurified ab109463 at 1/100. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

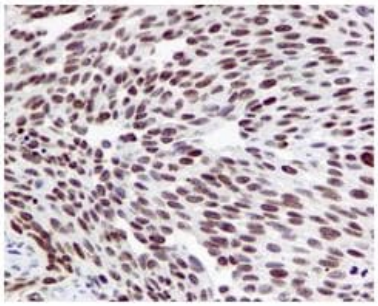
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon tissue labelling Histone H4 (acetyl K16) with purified ab109463 at 1/150. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human testis tissue labelling Histone H4 with unpurified ab109463 at 1/100.

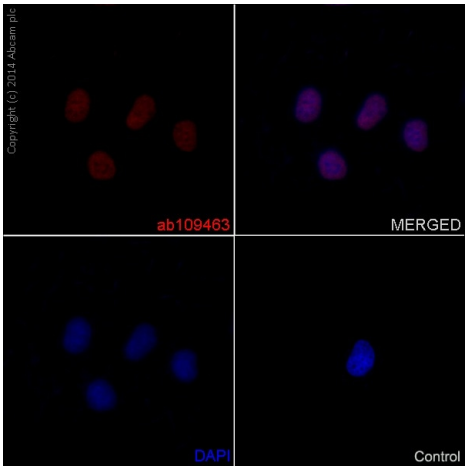
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human transitional cell carcinoma labelling Histone H4 (acetly K16) with unpurified ab109463 at 1/100.

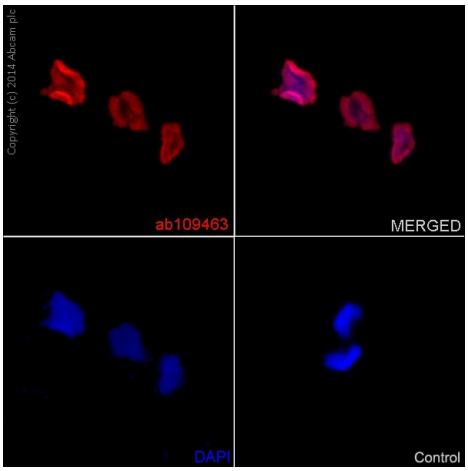
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling Histone H4 (acetyl K16) with unpurified ab109463 (red) at 1/100. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 555-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

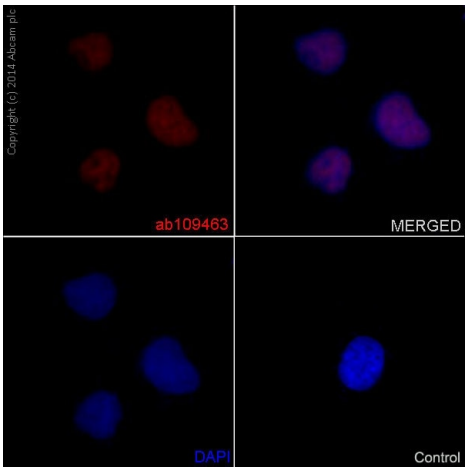
Control: primary antibody (1/100) and secondary antibody **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells treated with TSA labelling Histone H4 (acetyl K16) with unpurified ab109463 (red) at 1/100. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 555-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

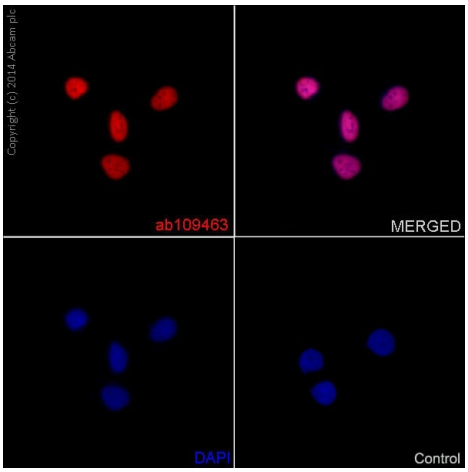
Control: primary antibody (1/100) and secondary antibody **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling Histone H4 (acetyl K16) with purified ab109463 (red) at 1/150. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 555-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

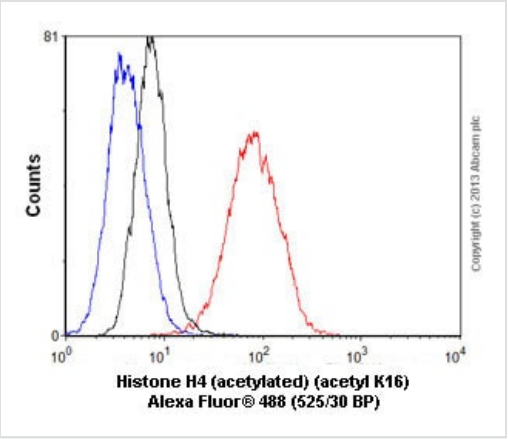
Control: primary antibody (1/150) and secondary antibody **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

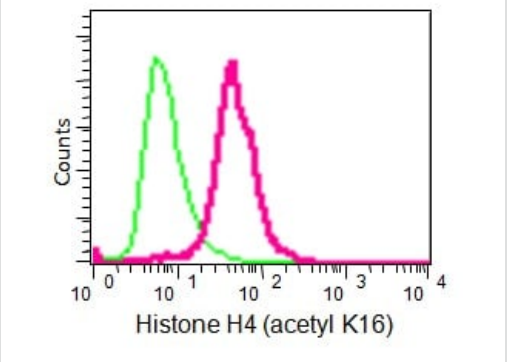
Immunocytochemistry/Immunofluorescence analysis of HeLa cells treated with TSA labelling Histone H4 (acetyl K16) with purified ab109463 (red) at 1/150. Cells were fixed with 4% paraformaldehyde. An Alexa Fluor® 555-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

Control: primary antibody (1/150) and secondary antibody **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/500).



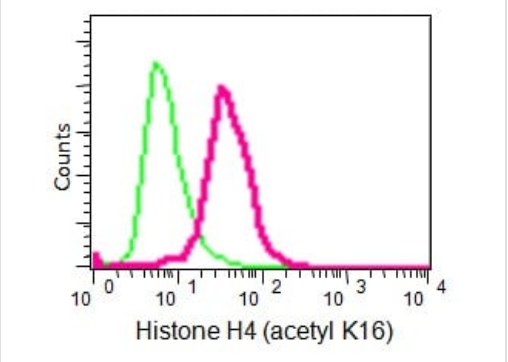
Flow Cytometry (Intracellular) - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Overlay histogram showing HeLa cells stained with unpurified ab109463 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab109463, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluorr® 488 goat anti-rabbit IgG (H+L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



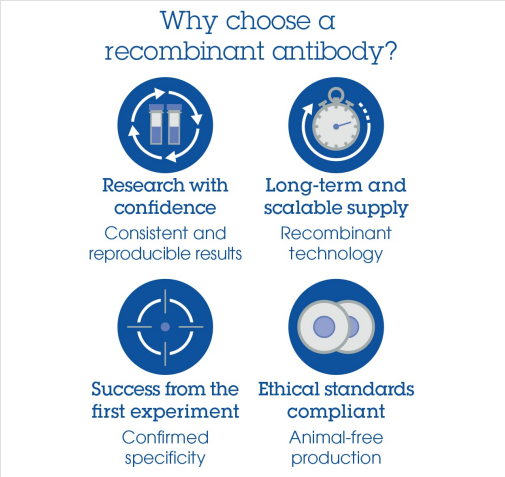
Flow Cytometry (Intracellular) - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Intracellular Flow Cytometry analysis of HeLa cells labelling Histone H4 (acetyl K16)with unpurified ab109463 (red) at 1/130. Cells were fixed with 80% methanol. A FITC-conjugated goat anti-rabbit IgG was used as the secondary antibody (1/150). A rabbit monoclonal IgG was used as the isotype control (green).



Flow Cytometry (Intracellular) - Anti-Histone H4 (acetyl K16) antibody [EPR1004] (ab109463)

Intracellular Flow Cytometry analysis of HeLa cells labelling Histone H4 (acetyl K16) with purified ab109463 (red) at 1/200. Cells were fixed with 80% methanol. A FITC-conjugated goat anti-rabbit IgG was used as the secondary antibody (1/150). A rabbit monoclonal IgG was used as the isotype control (green).



Anti-Histone H4 (acetyl K16) antibody [EPR1004]

(ab109463)

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