abcam

Product datasheet

Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade ab32129

Recombinant RabMAb

6 Abreviews 38 References 18 Images

Overview

Product name	Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade
Description	Rabbit monoclonal [Y28] to Histone H3 (acetyl K9) - ChIP Grade
Host species	Rabbit
Specificity	This antibody may be cross reactive with other acetyl sites such as k4, k14 and k18.
Tested applications	**Suitable for:** Flow Cyt (Intra), ChIP-sequencing, ChIC/CUT&RUN-seq, ChIP, WB, IHC-P, IP, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Histone H3 aa 1-100. The exact sequence is proprietary. Database link: **P68431**
Positive control	WB: HeLa (Human cervix adenocarcinoma epithelial cell), NIH/3T3 (Mouse embryonic fibroblast) and C6 (Rat glial tumor glial cell) treated with 500ng/ml Trichostatin A for 4 hours whole cell lysate IHC-P: Human cerebrum, colorectal carcinoma, mouse colon and rat colon tissue sections. ChIP: Chromatin prepared from HeLa cells. ChIP-seq: Chromatin prepared from HeLa cells. IP: HeLa cells. ICC/IF: HeLa cells. ChIC/CUT&RUN: 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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y28
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32129 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)		Use at an assay dependent concentration.
ChIP-sequencing		Use 4 µg for 30 µg of chromatin.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5 µg
ChIP		Use 2 µg for 25 µg of chromatin.
WB	★★★★★ (4)	1/500. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).
IHC-P	★★★★★ (1)	1/50 - 1/200. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		1/30.
ICC/IF	★★★★★ (1)	1/250.

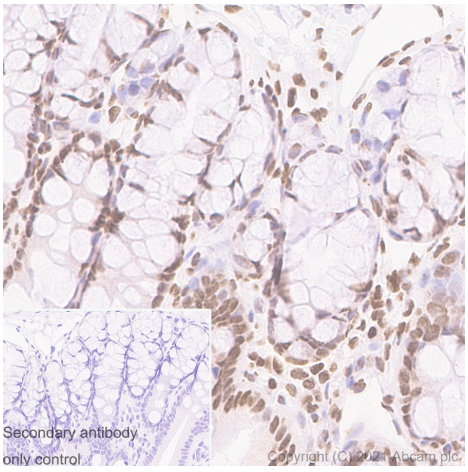
Target

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 H3 family.
Developmental stage	Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.
Post-translational modifications	Acetylation is generally linked to gene activation. Acetylation on Lys-10 (H3K9ac) impairs methylation at Arg-9 (H3R8me2s). Acetylation on Lys-19 (H3K18ac) and Lys-24 (H3K24ac) favors methylation at Arg-18 (H3R17me). Citrullination at Arg-9 (H3R8ci) and/or Arg-18 (H3R17ci) by PADI4 impairs methylation and represses transcription.

Asymmetric dimethylation at Arg-18 (H3R17me2a) by CARM1 is linked to gene activation. Symmetric dimethylation at Arg-9 (H3R8me2s) by PRMT5 is linked to gene repression. Asymmetric dimethylation at Arg-3 (H3R2me2a) by PRMT6 is linked to gene repression and is mutually exclusive with H3 Lys-5 methylation (H3K4me2 and H3K4me3). H3R2me2a is present at the 3' of genes regardless of their transcription state and is enriched on inactive promoters, while it is absent on active promoters.
Methylation at Lys-5 (H3K4me), Lys-37 (H3K36me) and Lys-80 (H3K79me) are linked to gene activation. Methylation at Lys-5 (H3K4me) facilitates subsequent acetylation of H3 and H4. Methylation at Lys-80 (H3K79me) is associated with DNA double-strand break (DSB) responses and is a specific target for TP53BP1. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are linked to gene repression. Methylation at Lys-10 (H3K9me) is a specific target for HP1 proteins (CBX1, CBX3 and CBX5) and prevents subsequent phosphorylation at Ser-11 (H3S10ph) and acetylation of H3 and H4. Methylation at Lys-5 (H3K4me) and Lys-80 (H3K79me) require preliminary monoubiquitination of H2B at 'Lys-120'. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are enriched in inactive X chromosome chromatin.
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and RPS6KA5 is important during interphase because it enables the transcription of genes following external stimulation, like mitogens, stress, growth factors or UV irradiation and result in the activation of genes, such as c-fos and c-jun. Phosphorylation at Ser-11 (H3S10ph), which is linked to gene activation, prevents methylation at Lys-10 (H3K9me) but facilitates acetylation of H3 and H4. Phosphorylation at Ser-11 (H3S10ph) by AURKB mediates the dissociation of HP1 proteins (CBX1, CBX3 and CBX5) from heterochromatin. Phosphorylation at Ser-11 (H3S10ph) is also an essential regulatory mechanism for neoplastic cell transformation. Phosphorylated at Ser-29 (H3S28ph) by MLTK isoform 1, RPS6KA5 or AURKB during mitosis or upon ultraviolet B irradiation. Phosphorylation at Thr-7 (H3T6ph) by PRKCBB is a specific tag for epigenetic transcriptional activation that prevents demethylation of Lys-5 (H3K4me) by LSD1/KDM1A. At centromeres, specifically phosphorylated at Thr-12 (H3T11ph) from prophase to early anaphase, by DAPK3 and PKN1. Phosphorylation at Thr-12 (H3T11ph) by PKN1 is a specific tag for epigenetic transcriptional activation that promotes demethylation of Lys-10 (H3K9me) by KDM4C/JMJD2C.
Phosphorylation at Tyr-42 (H3Y41ph) by JAK2 promotes exclusion of CBX5 (HP1 alpha) from chromatin.
Monoubiquitinated by RAG1 in lymphoid cells, monoubiquitination is required for V(D)J recombination (By similarity). 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.

Cellular localization Nucleus. Chromosome.

Images

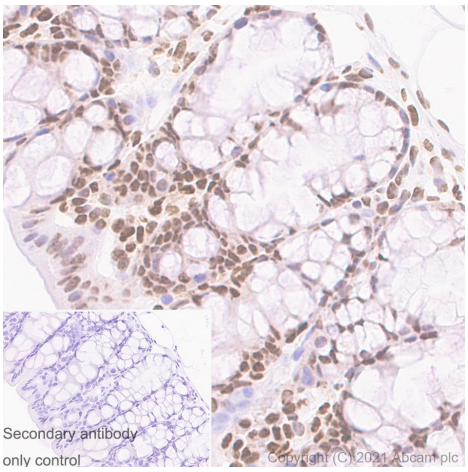


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129)

Immunohistochemical analysis of Paraffin-embedded sections rat colon tissue labelling Histone H3 (acetyl K9) with ab32129 at 1/6000 dilution, followed by a ready to use secondary Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Staining on rat colon tissue is observed. Counter stained with Haematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0).

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129)

Immunohistochemical analysis of Paraffin-embedded sections mouse colon tissue labelling Histone H3 (acetyl K9) with ab32129 at 1/6000 dilution, followed by a ready to use secondary Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Staining on mouse colon tissue is observed. Counter stained with Haematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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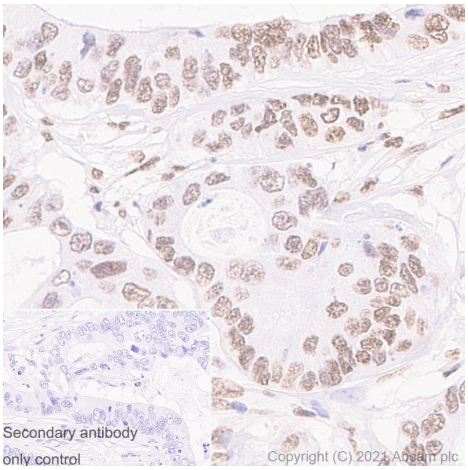

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129)

Immunohistochemical analysis of Paraffin-embedded sections human colorectal carcinoma tissue labelling Histone H3 (acetyl K9) with ab32129 at 1/2000 dilution, followed by a ready to use secondary Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Staining on human colorectal carcinoma tissue is observed. Counter stained with Haematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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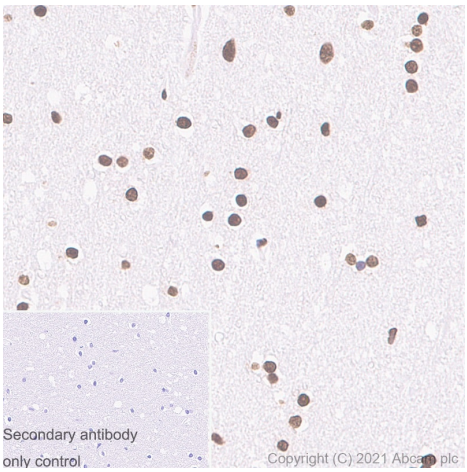

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129)

Immunohistochemical analysis of Paraffin-embedded sections human cerebrum tissue labelling Histone H3 (acetyl K9) with ab32129 at 1/2000 dilution, followed by a ready to use secondary Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Staining on human cerebrum tissue is observed. Counter stained with Haematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

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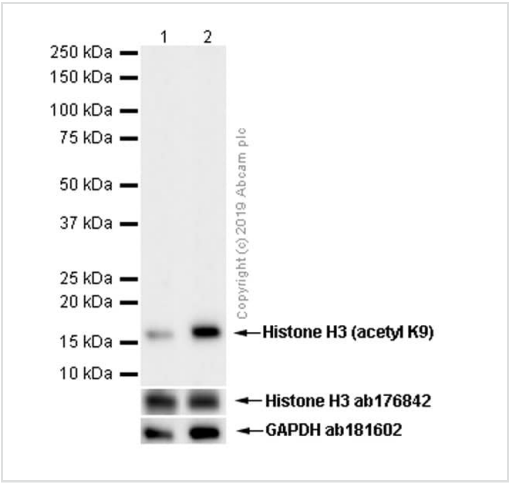


Western blot - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129)

All lanes : Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129) at 1/10000 dilution

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

Lysates/proteins at 15 µg per lane.

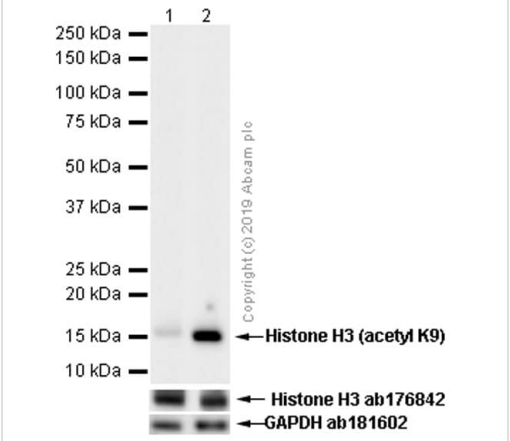
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 15 kDa
Observed band size: 15 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

ab181602 was used as GAPDH loading control.

ab176842 was for Histone H3 detection.



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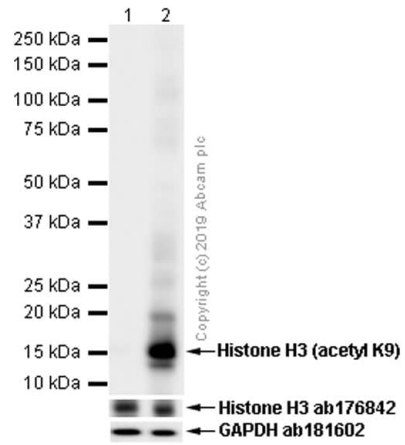

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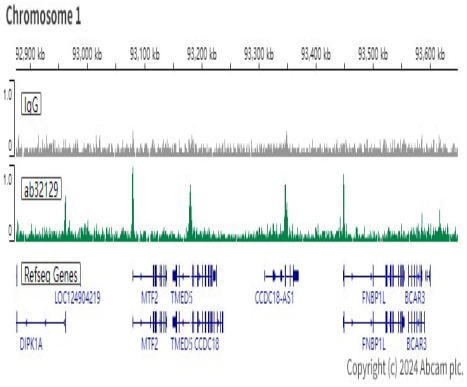
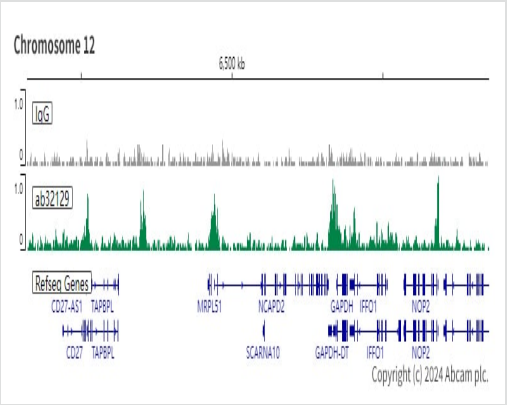


ChIC/CUT&RUN sequencing - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human epithelial cell line from cervix adenocarcinoma) cells and 5 µg of ab32129 [Y28]. 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.

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



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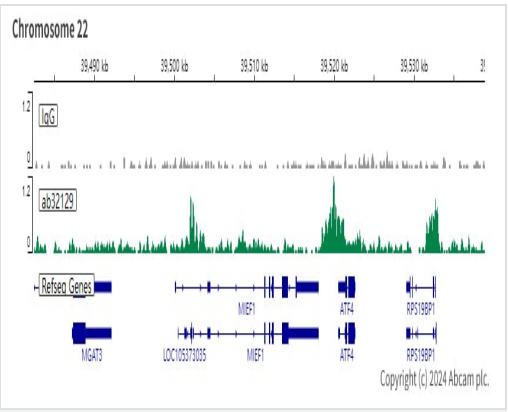

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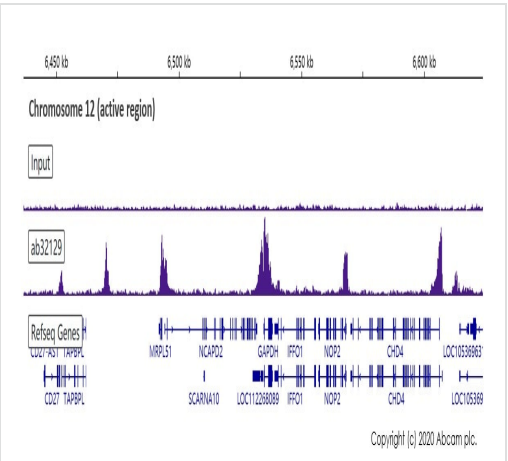
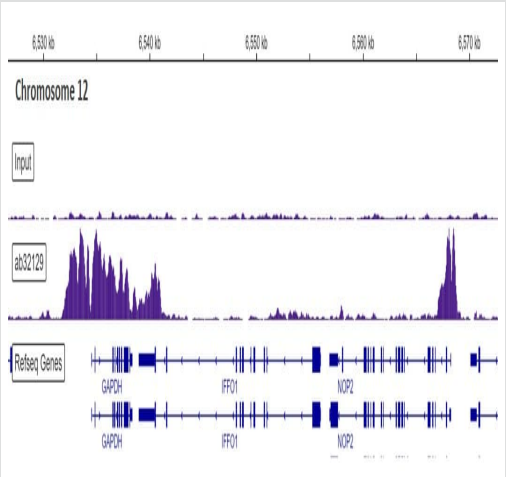


ChIP-sequencing - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129)

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 Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129). ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

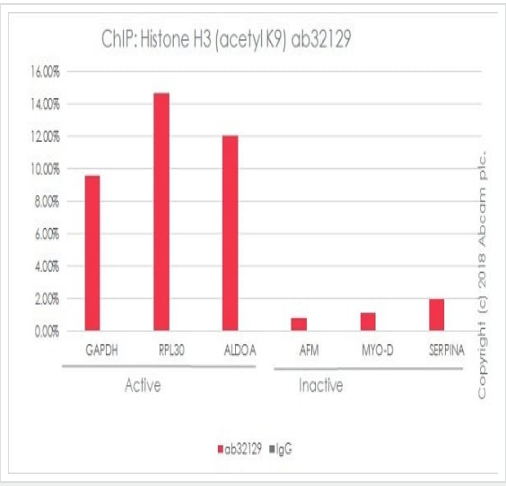
Additional screenshots of mapped reads can be downloaded **here**.



ChIP-sequencing - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129)

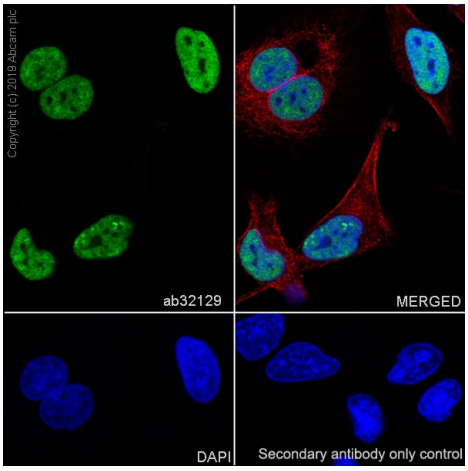
Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 30 µg of chromatin and 4 µg of Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129). ChIP DNA was sequenced on the Illumina NextSeq 500 to a depth of 30 million reads. ChIP-Seq validation performed by Active Motif, Carlsbad, CA.

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



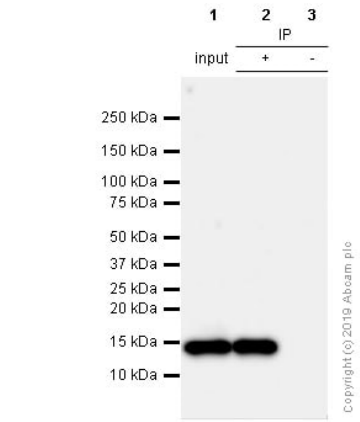
ChIP - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25 µg of chromatin, 2 µg of ab32129 (red), and 20 µl of Protein A/G sepharose beads. No antibody was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach).
Primers and probes are located in the first kb of the transcribed region.



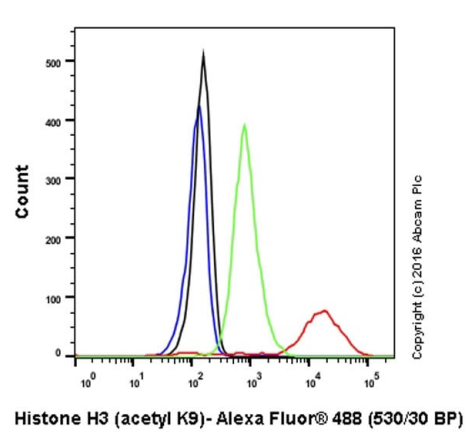
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (human cervix adenocarcinoma epithelial cell) cells labeling Histone H3 with purified ab32129 at 1/250 dilution (4 µg/mL). Cells were fixed in 100% Methanol and permeabilized with None. 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. **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/mL) was used as the secondary antibody only control.



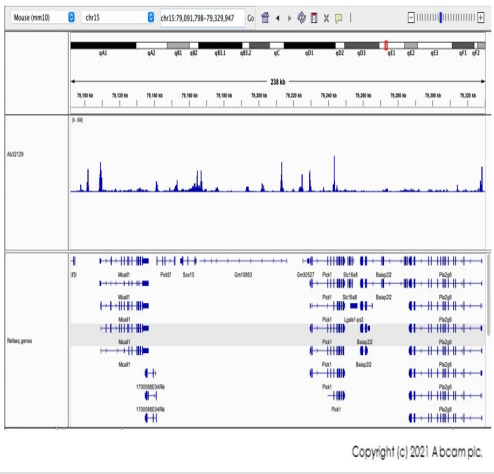
Immunoprecipitation - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129)

ab32129 (purified) at 1/30 dilution (20 µg/ml) immunoprecipitating Histone H3 acetyl K9 in TSA treated HeLa whole cell lysate.
Lane 1 (input): HeLa (human cervix adenocarcinoma epithelial cell) treated with 500ng/ml TSA for 4h whole cell lysate 10µg
Lane 2 (+): ab32129 & TSA treated HeLa whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32129 in TSA treated HeLa whole cell lysate
For western blotting, ab32129 at 1/500 and veriBlot for IP secondary antibody (HRP) (**ab131366**) was used at 1/1000 dilution. Blocking and diluting buffer: 5% NFDM /TBST.



Flow Cytometry (Intracellular) - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) treated (Red)/untreated (Green) with 500ng/ml Trichostatin A for 4 hours with purified ab32129 at 1/230 dilution. The secondary antibody was Goat anti rabbit IgG (Alexa Fluorr® 488) at 1/2000 dilution. A Rabbit monoclonal IgG (Black) was used as the isotype control and cells without incubation with primary antibody and secondary antibody (Blue) were used as unlabeled control.



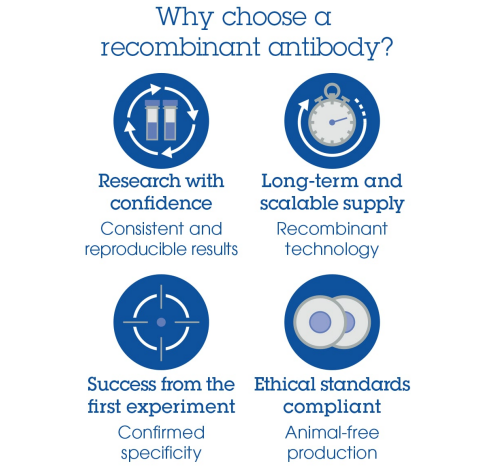
CUT&Tag sequencing - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129)

This experiment and image is courtesy of Dr Marek Bartosovic, Gonçalo Castelo-Branco Group, Karolinska Institutet.

CUT&Tag-seq was performed using 200,000 Oli-neu (Oligodendrocyte progenitor) cells. Cells were permeabilized with 0.05% Digitonin and 0.01% NP-40 for 3 minutes. A 1:100 dilution of Recombinant Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129) was used, along with a Guinea pig anti-rabbit Secondary. DNA was seg using Illumina NovaSeq S Prime to a depth of 24 million reads.

This image is courtesy of Dr Marek Bartosovic, Gonçalo Castelo-Branco Group, Karolinska Institutet.

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



Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129)

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