

Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free ab203951

Recombinant **RabMAb**

[17 Images](#)

Overview

Product name	Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free
Description	Rabbit monoclonal [Y28] to Histone H3 (acetyl K9) - ChIP Grade – BSA and Azide free
Host species	Rabbit
Specificity	This antibody may be cross reactive with other acetyl sites such as k4, k14 and k18.
Tested applications	Suitable for: ChIC/CUT&RUN-seq, Flow Cyt (Intra), ChIP-sequencing, IP, IHC-P, ICC/IF, ChIP, WB
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
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	<p>ab203951 is the carrier-free version of ab32129.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - 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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y28
Isotype	IgG

Applications

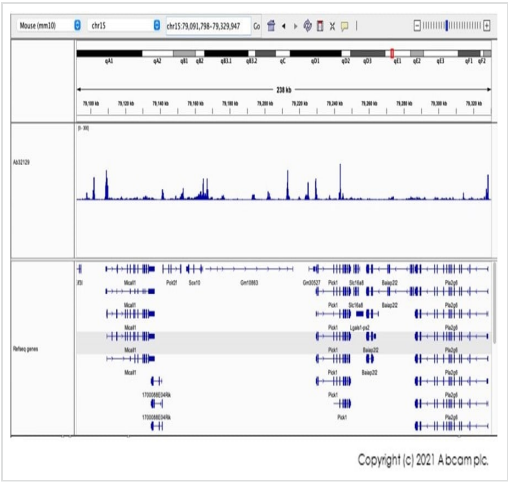
The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab203951 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5 µg
Flow Cyt (Intra)		Use at an assay dependent concentration.
ChIP-sequencing		Use 4 µg for 30 µg of chromatin.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
ChIP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).

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	<p>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).</p> <p>Citrullination at Arg-9 (H3R8ci) and/or Arg-18 (H3R17ci) by PAD4 impairs methylation and represses transcription.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>Phosphorylation at Tyr-42 (H3Y41ph) by JAK2 promotes exclusion of CBX5 (HP1 alpha) from chromatin.</p> <p>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.</p>

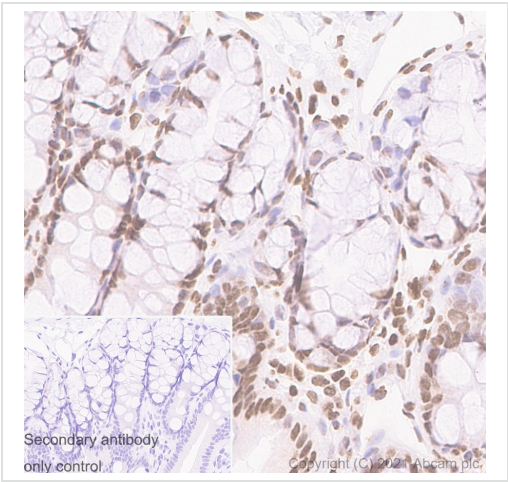
Images



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 seq using Illumina NovaSeq S Prime to a depth of 24 million reads.

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

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

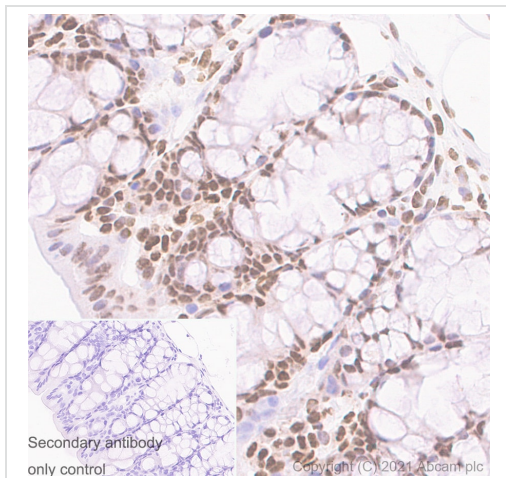


This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32129**).

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 - BSA and Azide free (ab203951)

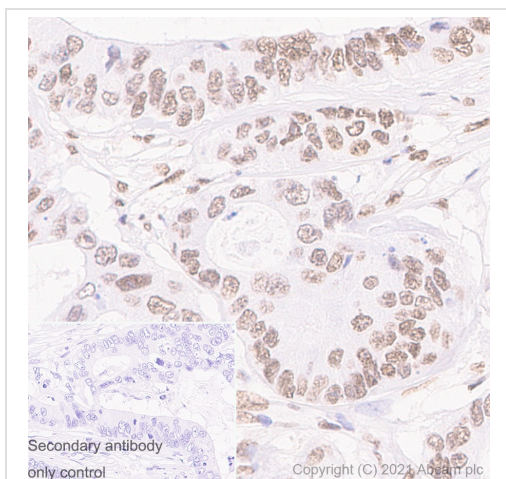
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Immunohistochemical analysis of Paraffin-embedded sections mouse colon tissue labelling Histone H3 (acetyl K9) with [ab32129](#) at 1/6000 dilution, followed by a ready to use secondary Rabbit specific IHC polymer detection kit HRP/DAB ([ab209101](#)). 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 - BSA and Azide free (ab203951)

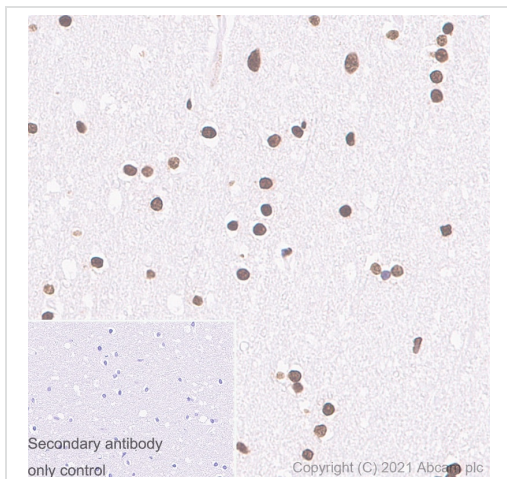
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab32129](#)).

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](#)).

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 - BSA and Azide free (ab203951)

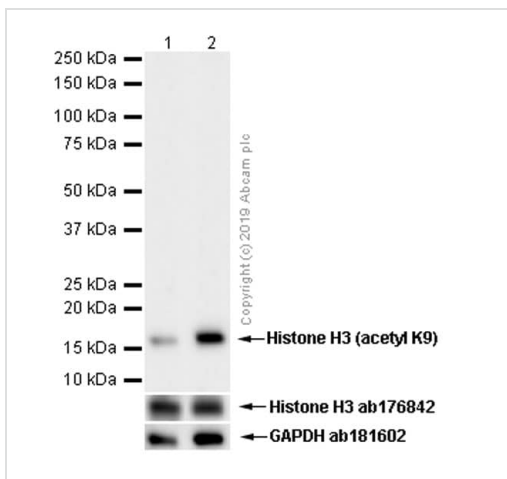
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab32129](#)).

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](#)).

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

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



Western blot - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab32129](#)).

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

[ab181602](#) was used as GAPDH loading control.

ab176842 was for Histone H3 detection.

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

Lane 1 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) treated with 500ng/ml Trichostatin A for 4 hours whole cell lysate

Lysates/proteins at 15 µg per lane.

Secondary

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ab181602 was used as GAPDH loading control.

ab176842 was for Histone H3 detection.

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

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) treated with 500ng/ml Trichostatin A for 4 hours whole cell lysate

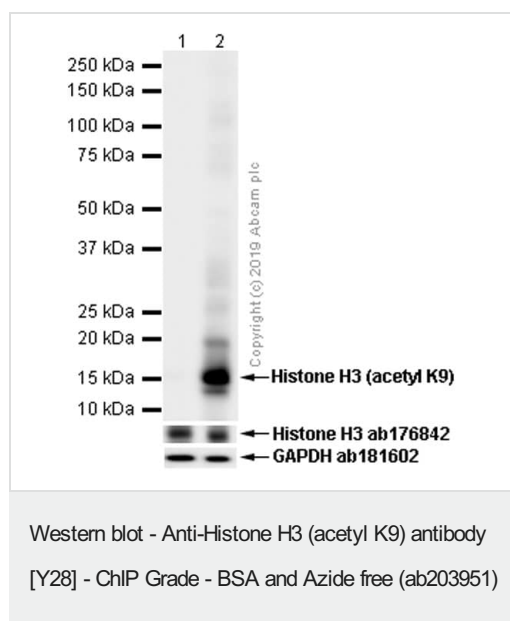
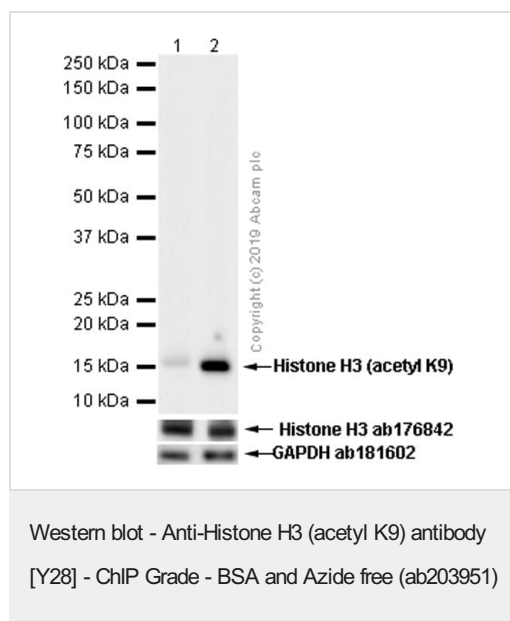
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Secondary

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Predicted band size: 15 kDa

Observed band size: 15 kDa



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

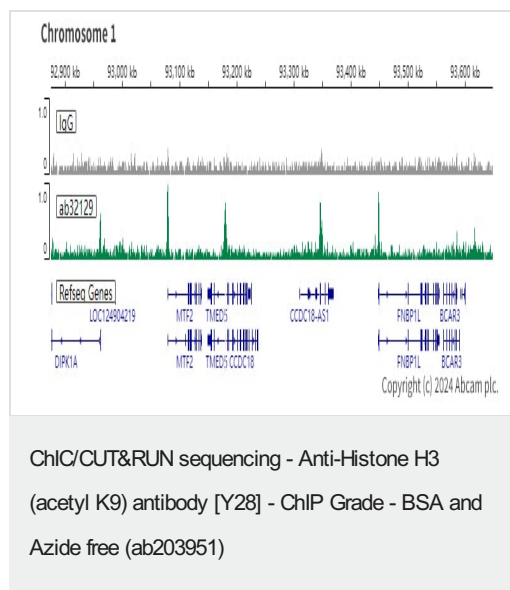
ab181602 was used as GAPDH loading control.

ab176842 was for Histone H3 detection.

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

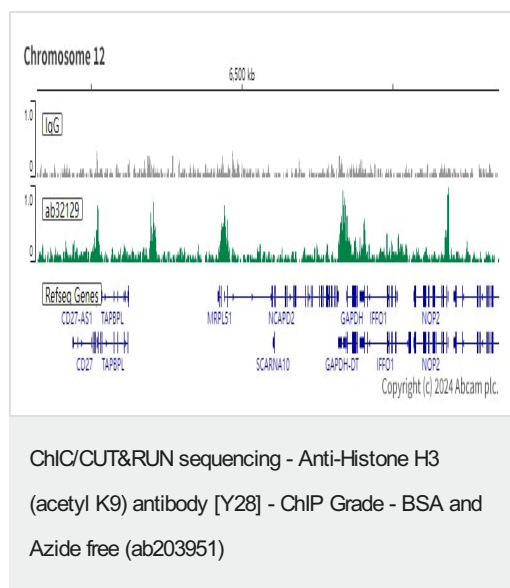
This data was developed using the same antibody clone in a different buffer formulation (**ab32129**).

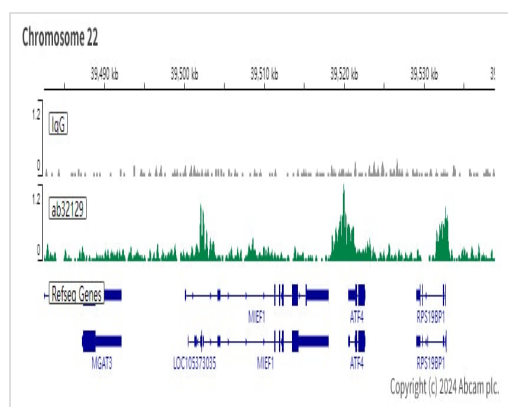


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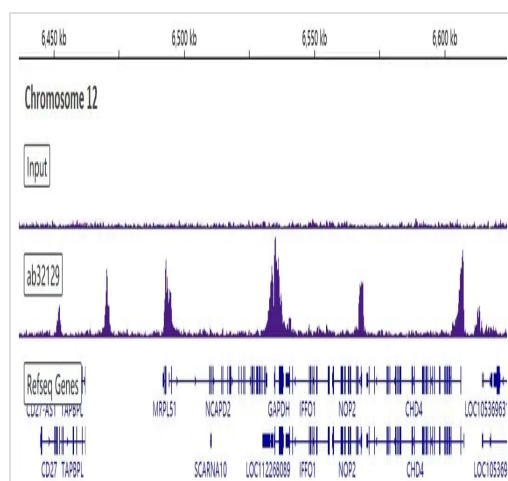


ChIC/CUT&RUN sequencing - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

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

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This data was developed using the same antibody clone in a different buffer formulation (**ab32129**).

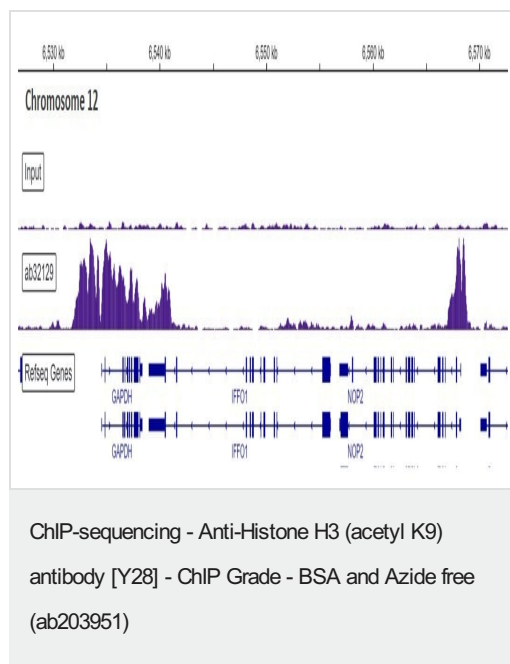


ChIP-sequencing - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

This data was developed using the same antibody clone in a different buffer formulation (**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 μ g 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.

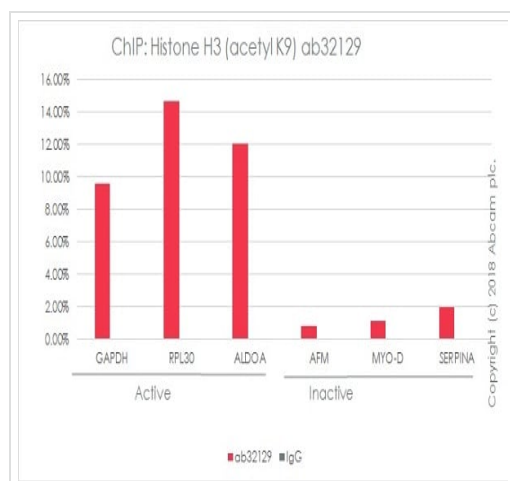
Additional screenshots of mapped reads can be downloaded [here](#).



This data was developed using the same antibody clone in a different buffer formulation ([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.

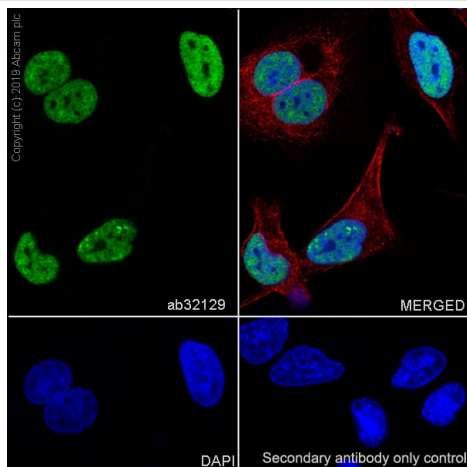
Additional screenshots of mapped reads can be downloaded [here](#).



ChIP - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

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 (gray). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach). Primers and probes are located in the first kb of the transcribed region.

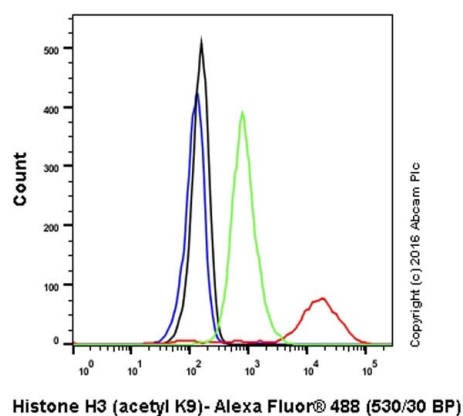
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab32129](#)).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

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% Methano. 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32129**).



Flow Cytometry (Intracellular) - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

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 Fluor® 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32129**).

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP
Grade - BSA and Azide free (ab203951)

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