abcam

Product datasheet

Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade ab213224

Recombinant RabMAb

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Overview

Product name	Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade
Description	Rabbit monoclonal [EPR20551-225] to Histone H3 (tri methyl K4) - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** ChIP-sequencing, Flow Cyt (Intra), ChIP, WB, ICC/IF, Dot blot, PepArr, IP, ChIC/CUT&RUN-seq
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, NIH/3T3 and C6 whole cell lysates. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt (intra): HeLa cells. IP: NIH/3T3 whole cell lysate. ChIP: Chromatin prepared from HeLa cells. ChIP-seq: Chromatin prepared from HeLa cells. Dot blot: Histone H3K4Me3 peptide. 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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), PBS
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR20551-225
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab213224 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
ChIP-sequencing		Use 4 µg for 30 µg of chromatin.
Flow Cyt (Intra)		1/500.
ChIP	★★★★★ (1)	Use 2 µg for 25 µg of chromatin.
WB	★★★★☆ (4)	1/1000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa). We recommend to use 2% BSA as blocking and antibody dilution buffer.
ICC/IF		1/500.
Dot blot		1/1000.
PepArr		Use a concentration of 0.1 µg/ml.
IP		1/30.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 2 µg

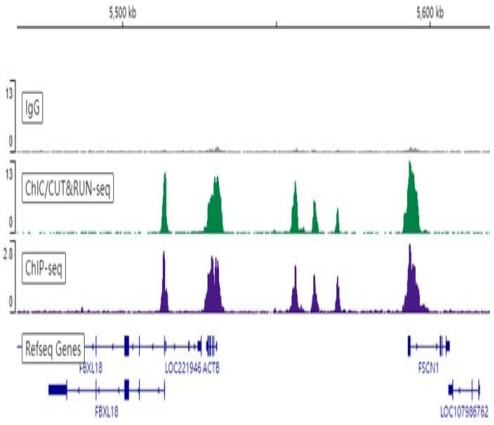
Target

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



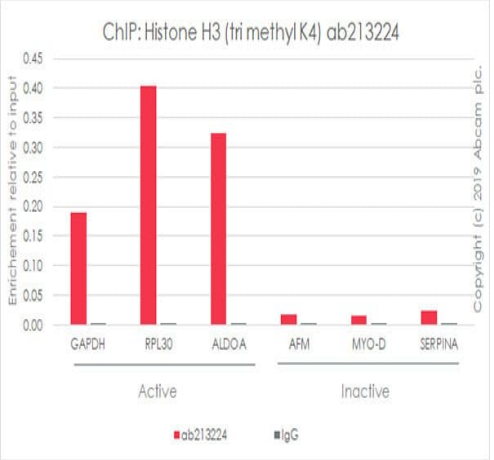
ChIC/CUT&RUN sequencing - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

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 ab213224 [EPR20551-225]. 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 ChIP data was conducted on chromatin 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 ab213224. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

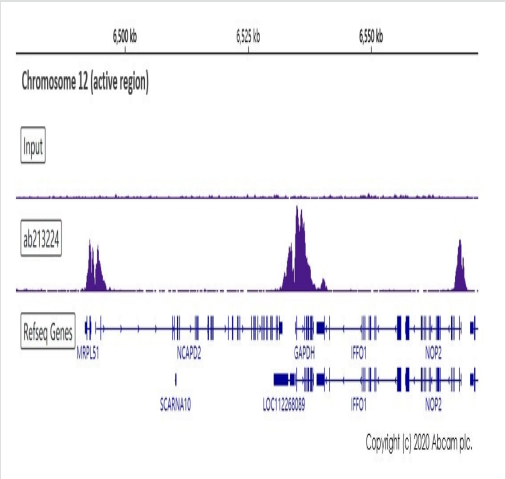
Additional screenshots of mapped reads can be downloaded **here**.

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



ChIP - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

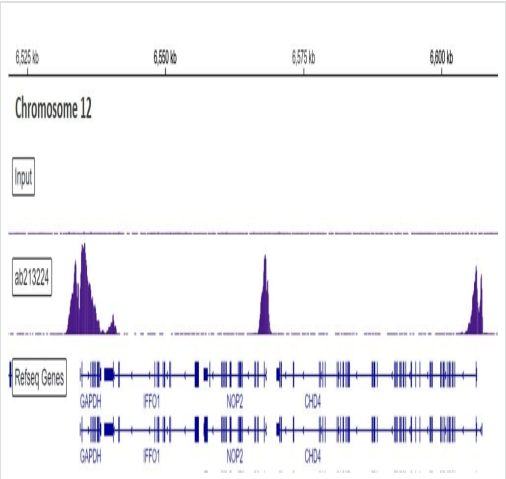
Chromatin was prepared from Hela (human epithelial cel line from cervix adenocarcinoma) cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 min. The ChIP was performed with 25 µg of chromatin, 2 µg of ab213224(red), and 20 µl of Anti-rabbit IgG sepharose beads. 2 µg of Rabbit normal IgG 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.



ChIP-sequencing - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 cells and 4 µg of Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

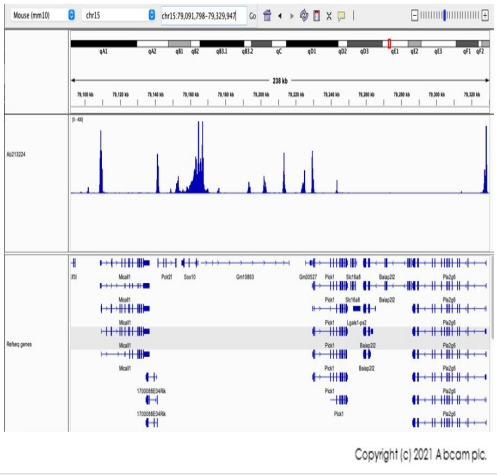
Additional screenshots of mapped reads can be downloaded **here**.



ChIP-sequencing - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

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 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade. 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**.



CUT&Tag sequencing - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

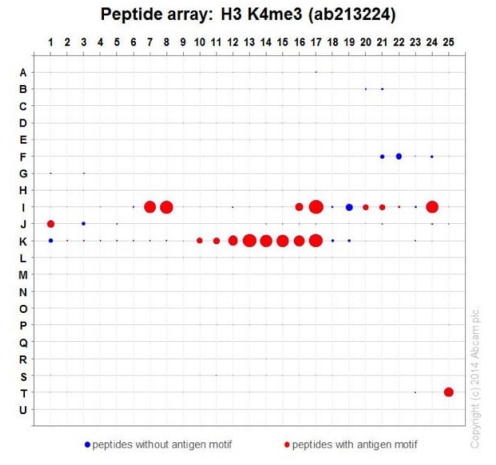
This experiment and image is courtesy of Dr Marek Bartosovic, Gonçalo Castelo-Branco Group, Karolinska Institutet.

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 Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade was used, along with a Guinea pig anti-rabbit Secondary. DNA was seg using Illumina NovaSeq S Prime to a depth of 22 million reads.

Positive control Sox10 chr15:79,091,798-79,329,947 (Mouse mm10 genome used).

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.

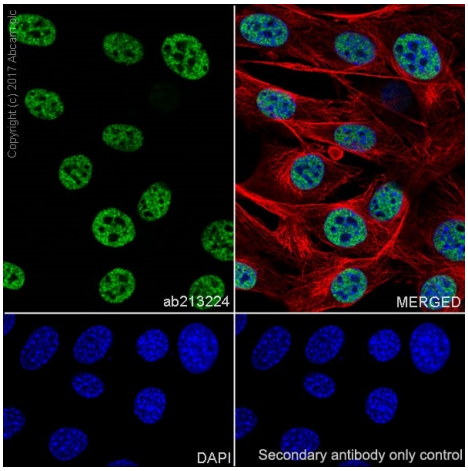


Peptide Array - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

ab213224 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**.

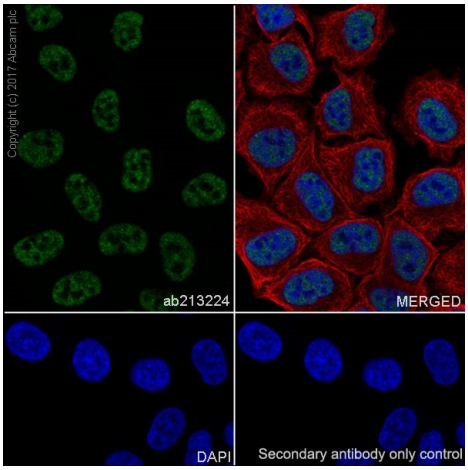


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1%, Triton X-100-permeabilized NIH/3T3 (mouse embryo fibroblast cell line) cells labeling Histone H3 (tri methyl K4) with ab213224 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Positive nuclear staining on NIH/3T3 cell lines.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

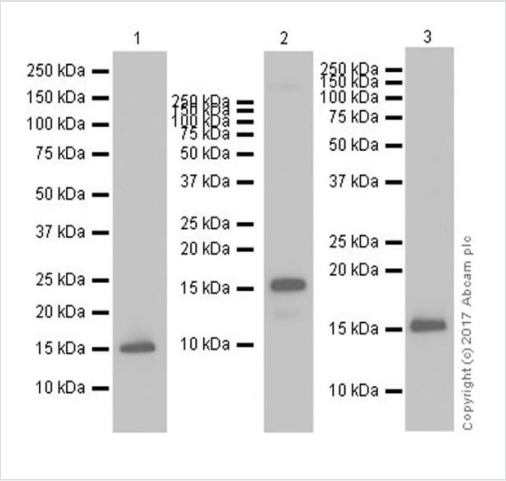


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100-permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling Histone H3 (tri methyl K4) with ab213224 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Positive nuclear staining on HeLa cell line.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Western blot - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

All lanes : Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224) at 1/5000 dilution

Lane 1 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate
Lane 3 : C6 (rat glial tumor cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

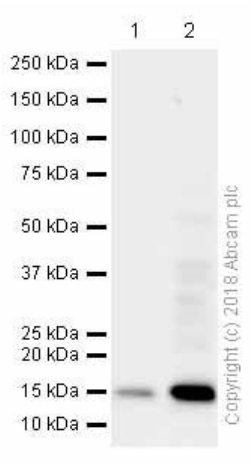
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Developed using the ECL technique.

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

Exposure time: 1 second

Blocking/Dilution buffer: 5% BSA/TBST.



Western blot - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

All lanes : Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224) at 1/5000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates with 2% BSA/TBST

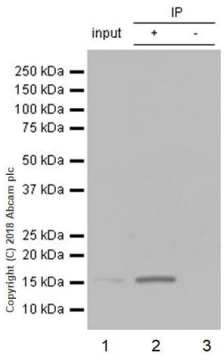
Lysates/proteins at 15 µg per lane.

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

Predicted band size: 15 kDa

Exposure time: 40 seconds

We recommend to use 2% BSA as blocking and antibody dilution buffer.



Immunoprecipitation - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

Histone H3 (tri methyl K4) was immunoprecipitated from 0.35 mg of NIH/3T3 (mouse embryo fibroblast cell line) lysate with ab213224 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab213224 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/5000 dilution

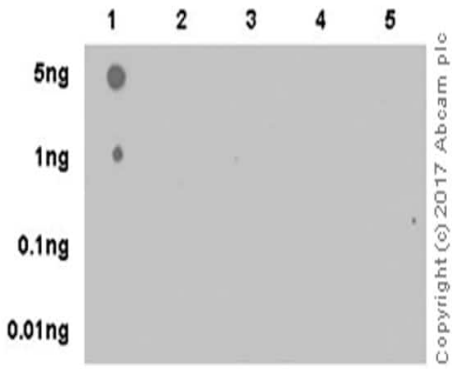
Lane 1: NIH/3T3 whole cell lysate (Input).

Lane 2: NIH/3T3 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab213224 in NIH/3T3 whole cell lysate.

Exposure time: 3 minutes.

Blocking and dilution buffer and concentration: 5% BSA/TBST.



Dot Blot - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

Dot blot analysis of Histone H3 (tri methyl K4) labeled with ab213224 at 1/1000 dilution.

Lane 1: Histone H3K4Me3 peptide.

Lane 2: Histone H3 unmodified peptide.

Lane 3: Histone H3K(18+K36)Me3 peptide.

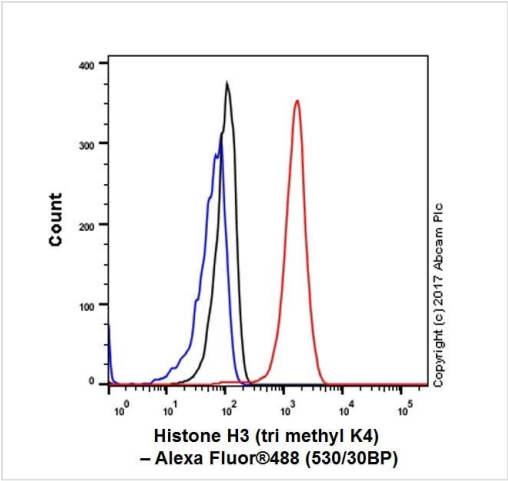
Lane 4: Histone H3K18Me3 peptide.

Lane 5: Histone H3K4Me2 peptide.

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution was used as secondary antibody.

Exposure time: 3 minutes.

Blocking and dilution buffer: 5% NFDM/TBST.



Flow Cytometry (Intracellular) - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling Histone H3 (tri methyl K4) with ab213224 at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

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