abcam

ab213224

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

Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224) is a rabbit monoclonal antibody detecting Histone H3 in **Western Blot, Flow Cytometry (Intra), IP, ICC/IF, ChIP, Dot Blot, PepArr**. Suitable for **Human, Mouse, Rat**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 20 publications

Recombinant | RabMAb | Advanced Validation | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogens	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR20551-225
Purification technique	Affinity purification Protein A
Specificity	We recommend to use 2% BSA as blocking and antibody dilution buffer.
Concentration	0.49 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

CUT&Tag

Tested

Species	Mouse
Dilution info	1/100
Notes	-

Expected

Species	Human, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Flow Cyt (Intra)

Expected

Species	Human
Dilution info	1/500
Notes	-

Species	Mouse
Dilution info	1/500
Notes	-

Species	Rat
Dilution info	1/500
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

ChIC/CUT&RUN-seq

Tested

Species	Human
Dilution info	2 µg
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

IP

Tested

Species	Mouse
Dilution info	1/30
Notes	-

Expected

Species	Human, Rat
Dilution info	Use at an assay dependent concentration.

Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

ChIP

Tested

Species	Human
Dilution info	2 µg for 25 µg chromatin
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Dot

Tested

Species	Synthetic peptide
Dilution info	1/1000
Notes	-

Expected

Species	Mouse, Human, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	We recommend to use 2% BSA as blocking and antibody dilution buffer.

Species	Rat
Dilution info	1/1000
Notes	We recommend to use 2% BSA as blocking and antibody dilution buffer.

Species	Human
Dilution info	1/1000
Notes	We recommend to use 2% BSA as blocking and antibody dilution buffer.

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

PepArr

Tested

Species	Synthetic peptide
Dilution info	0.1 µg/mL
Notes	-

Expected

Species	Mouse, Human, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Species	Human
Dilution info	1/500
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

ChIP-seq

Tested

Species	Human
Dilution info	4 µg for 30 µg chromatin
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Target data

See full target information H3C1 tri methyl K4

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.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Product Specifications
Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224) is a rabbit recombinant monoclonal antibody and is validated for use in CUT&Tag, ChIC/CUT&RUN-seq, ChIP, ChIP-seq, Dot Blot, Flow Cyt (Intra), ICC/IF, IP, PepArr, WB in human, mouse and rat samples.
Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224) specifically detects Histone H3 Tri Methyl-K4 (UniProt ID: P68431; Molecular weight: 15kDa) and is sold in 100 µL and 1 mL selling sizes.

Quality and Validation
Abcam's high quality manufacturing and validation processes ensure Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.
Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224) has been cited over 22 times in peer reviewed journals and is trusted by the scientific community.
Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224) has 5 independent reviews from customers.

Related Products
Conjugation-ready, carrier free format available for antibody clone EPR20551-225 - ab313500.
Antibody clone EPR20551-225 is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, Alexa Fluor® 647, PE (ab237340, ab237341, ab237342).

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

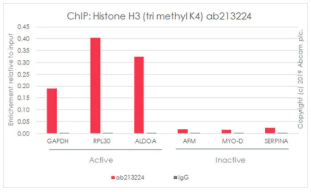
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

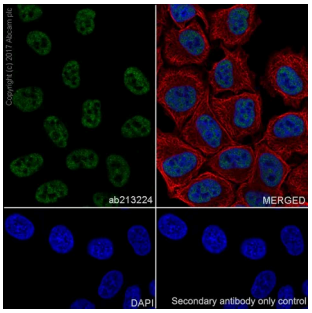
Full details and terms and conditions can be found here:
Terms & Conditions.

14 product images



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.

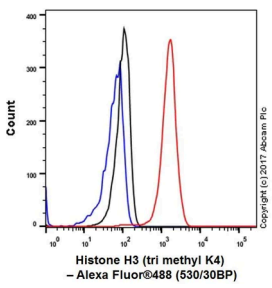


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

Histone H3 (tri methyl K4) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Histone H3 (tri methyl K4) antibody

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.

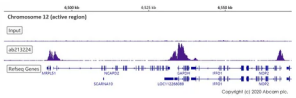


Flow Cytometry (Intracellular) - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

Histone H3 (tri methyl K4) Flow Cytometry (Intracellular) staining using rabbit Anti-Histone H3 (tri methyl K4) antibody

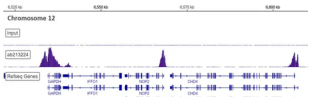
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling Histone H3 (tri methyl K4) with ab213224 at 1/500 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (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.



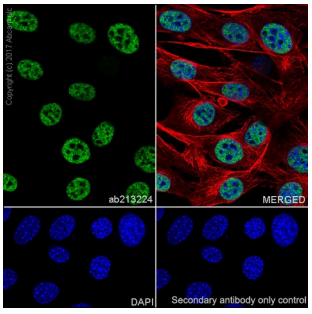
ChIP-sequencing - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 107 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.

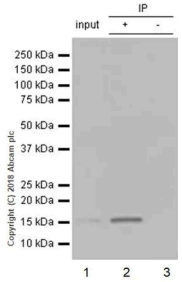


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

Histone H3 (tri methyl K4) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Histone H3 (tri methyl K4) antibody

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.



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.

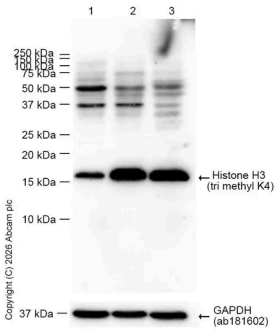
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab213224 in NIH/3T3 whole cell lysate.

Exposure time: 3 minutes.

Blocking and dilution buffer and concentration: 5% BSA/TBST.
All lanes:
Immunoprecipitation - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224)

Predicted band size: 15 kDa

Observed band size: 15 kDa



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

Histone H3 (tri methyl K4) Western blot staining using rabbit Anti-Histone H3 (tri methyl K4) antibody

We recommend to use 2% BSA/TBST as blocking and antibody dilution buffer.
In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/1000000 dilution.

All lanes:
Western blot - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224) at 1/1000 dilution

Lane 1:
Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate at 20 µg

Lane 2:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

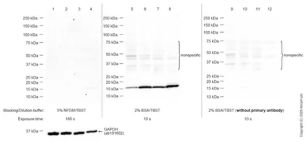
Lane 3:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/20000 dilution

Observed band size: 15 kDa, 36 kDa

Exposure time: 40s



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

Histone H3 (tri methyl K4) Western blot staining using rabbit Anti-Histone H3 (tri methyl K4) antibody

In Western blot, anti-GAPDH antibody (ab181602) loading control staining at 1/1000000 dilution.

Blocking and diluting buffer and concentration: Lane 1-4: 5% NFDM/TBST Lane 5-12: 2% BSA/TBST
We recommend using 2% BSA as blocking and antibody dilution buffer.

All lanes:
Western blot - Anti-Histone H3 (tri methyl K4) antibody [EPR20551-225] - ChIP Grade (ab213224) at 1/1000 dilution

Lanes 1, 5 and 9:

Raji (human Burkitt's lymphoma B lymphocyte) whole cell lysate at 20 µg

Lanes 2, 6 and 10:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

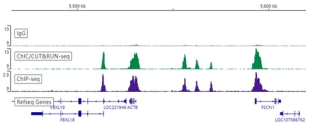
Lanes 3, 7 and 11:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

Lanes 4, 8 and 12:
C6 (rat glial tumor glial cell) whole cell lysate at 20 µg

Secondary

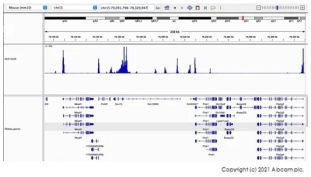
All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/20000 dilution

Exposure time: 40s



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

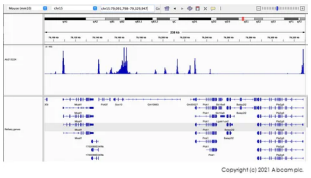
ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and 2 µg of ab213224 [EPR20551-225]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG 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 found in the Protocol booklet in the Product Protocol section. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



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

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

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.



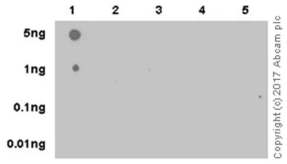
This experiment and image is courtesy of Dr Marek Bartosovic, Gonçalo Castelo-Branco

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

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).

Group, Karolinska Institutet.

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



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

Histone H3 (tri methyl K4) Dot Blot staining using rabbit Anti-Histone H3 (tri methyl K4) antibody

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.

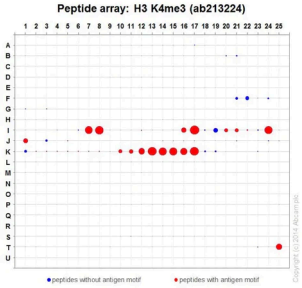
Lane 4: Histone H3K18Me3 peptide.

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.



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, is available under the Product Protocol section.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.