abcam

Product datasheet

Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] ab256384

Recombinant RabMAb

10 Images

Overview

Product name	Anti-Histone H3 (mutated K36M) antibody [EPR23614-91]
Description	Rabbit monoclonal [EPR23614-91] to Histone H3 (mutated K36M)
Host species	Rabbit
Tested applications	**Suitable for:** ChIC/CUT&RUN-seq, Flow Cyt (Intra), IP, WB, IHC-P, ICC/IF, Indirect ELISA **Unsuitable for:** ChIP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK-293 transfected with Histone H3.3 K36M (mutate) expression vector containing a myc-His-tag, whole cell lysate IHC-P: Human chondroblastoma tissue. ICC/IF: HEK-293 cells Flow Cyt (intra): 293T transfected with myc tagged Histone H3 construct cell. IP: HEK-293 transfected with Histone H3.3 K36M cell. ChIC/CUT&RUN-Seq: 293T cells transfected with a H3.3K36M (Mutant) plasmid.
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: 0.05% Sodium azide Constituents: PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR23614-91
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab256384 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. 3 µg
Flow Cyt (Intra)		1/500.
IP		1/30.
WB		1/1000. Predicted molecular weight: 15 kDa.
IHC-P		1/4000. Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0)
ICC/IF		1/50.
Indirect ELISA		Use a concentration of 1 µg/ml.

Application notes Is unsuitable for ChIP.

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.

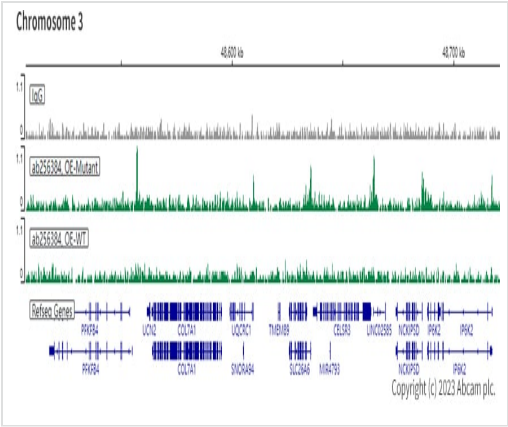
Developmental stage Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

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

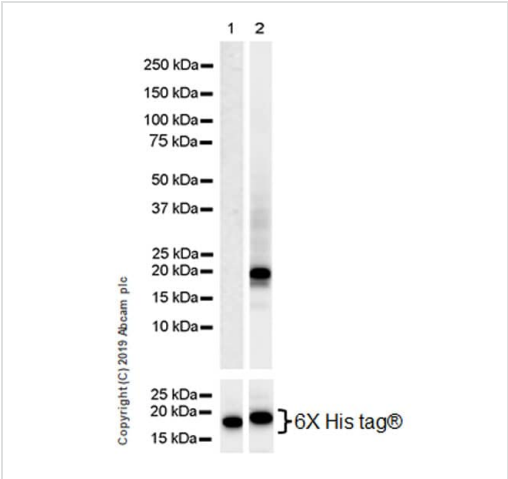


ChIC/CUT&RUN sequencing - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] (ab256384)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 293T cells transfected with a H3.3K36M (Mutant) or H3.3 (WT) plasmid and 3µg of ab256384 [EPR23614-91]. 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 H3 (mutated K36M) antibody [EPR23614-91] (ab256384)

All lanes : Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] (ab256384) at 1/1000 dilution

Lane 1 : HEK-293 transfected with Histone H3.3 (WT) expression vector containing a myc-His-tag®, whole cell lysate
Lane 2 : HEK-293 transfected with Histone H3.3 K36M (mutate) expression vector containing a myc-His-tag®, whole cell lysate

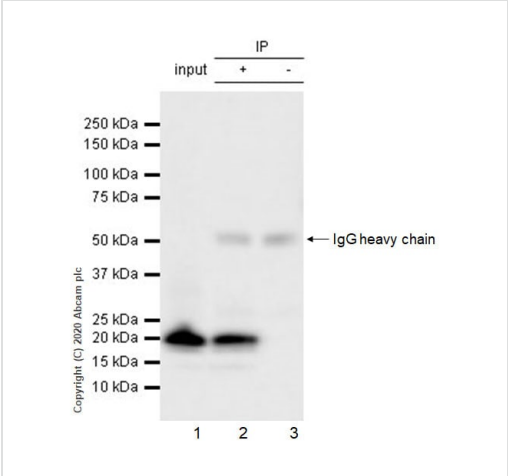
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 15 kDa
Observed band size: 20 kDa

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

Exposure time: 26 seconds



Immunoprecipitation - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] (ab256384)

Histone H3.3 (mutated K36 M) was immunoprecipitated from 0.35 mg HEK-293 transfected with Histone H3.3 K36M (mutate) expression vector containing a myc-His-tag®, whole cell lysate with ab256384 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab256384 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

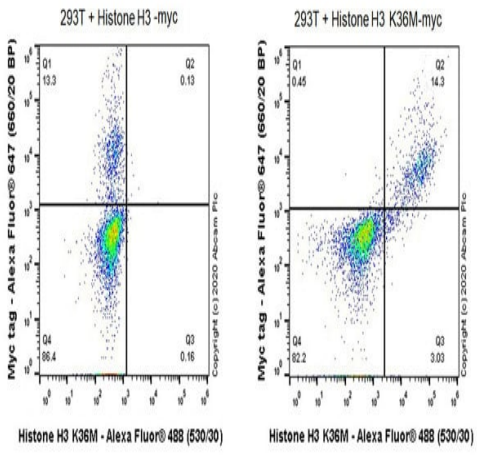
Lane 1: HEK-293 transfected with Histone H3.3 K36M (mutate) expression vector containing a myc-His-tag®, whole cell lysate 10 ug

Lane 2: ab256384 IP in HEK-293 transfected with Histone H3.3 K36M (mutate) expression vector containing a myc-His-tag®, whole cell lysate

Lane 3:Rabbit monoclonal IgG (**ab172730**) instead of ab256384 in HEK-293 transfected with Histone H3.3 K36M (mutate) expression vector containing a myc-His-tag®, whole cell lysate

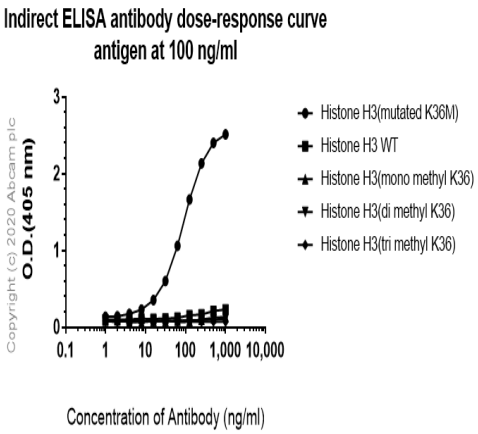
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds



Flow Cytometry (Intracellular) - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] (ab256384)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HEK-293T transfected with myc tagged Histone H3 construct (Left) or myc tagged Histone H3 K36M construct (Right) cells labelling Histone H3 (mutated K36 M) with ab256384 at 1/500 compared with a isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 was used as the secondary antibody.



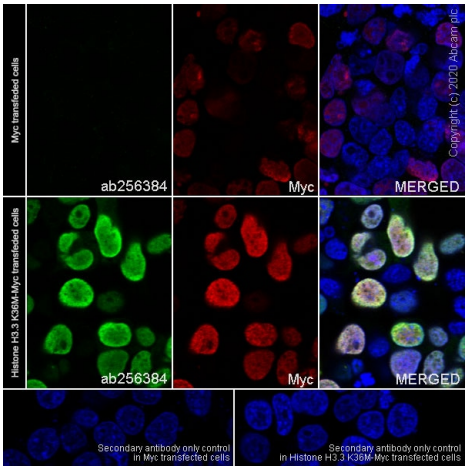
Indirect ELISA - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] (ab256384)

Indirect ELISA using ab256384 at varying antibody concentrations and antigen concentration at 100 ng/mL. An Alkaline Phosphatase-conjugated Goat Anti-Rabbit IgG (H+L) (1/2500) was used as the secondary antibody.

Binding was seen for Histone H3 (mutated K36M) peptide. Binding to the following peptides was not seen:

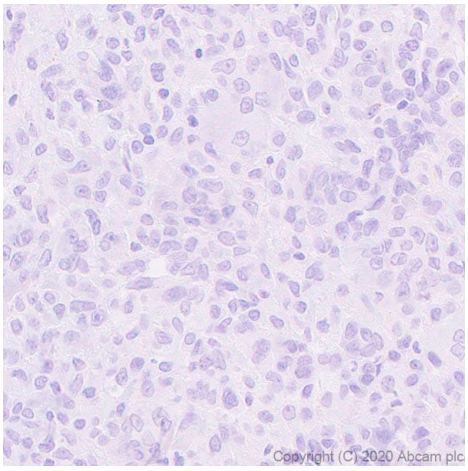
Histone H3 WT,
Histone H3 (mono methyl K36),
Histone H3 (di methyl K36),
Histone H3 (tri methyl K36).

This indicates the specificity of ab256384 for mutated K36M of Histone H3.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] (ab256384)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293 cells labelling Histone H3 (mutated K36 M) with ab256384 at 1/50 dilution, followed by a secondary antibody at 1/1000 dilution (Green). Confocal image showing nuclear staining in HEK-293 cell line transfected with myc-tagged Histone H3 K36M expression vector. 2233S Myc-Tag (9B11) Mouse mAb (Alexa Fluor® 647 Conjugate) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

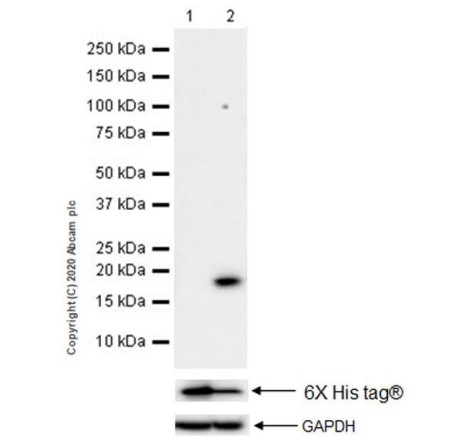


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] (ab256384)

Immunohistochemical analysis of paraffin-embedded human giant cell tumor of bone tissue labeling Histone H3 (mutated K36 M) with ab256384 at 1/4000 dilution followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). **Negative control:** No staining in human giant cell tumor of bone (PMID: 29757500). Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0)



Western blot - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] (ab256384)

All lanes : Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] (ab256384) at 1/1000 dilution

Lane 1 : HEK-293 transfected with Histone H3.1 (WT) expression vector containing a myc-His-tag®, whole cell lysate
Lane 2 : HEK-293 transfected with Histone H3.1 K36M (mutant) expression vector containing a myc-His-tag®, whole cell lysate

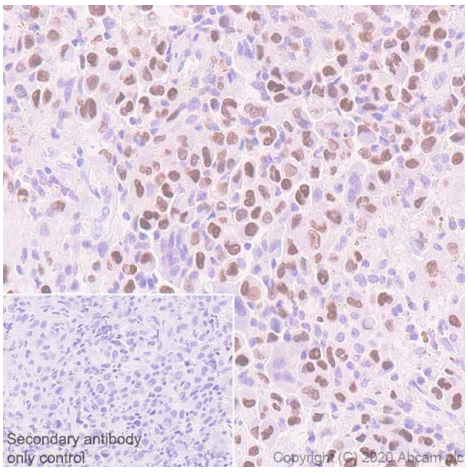
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 15 kDa
Observed band size: 19 kDa

Exposure time: 62 seconds

Blocking/Diluting buffer and concentration: 5% NFDM/TBST

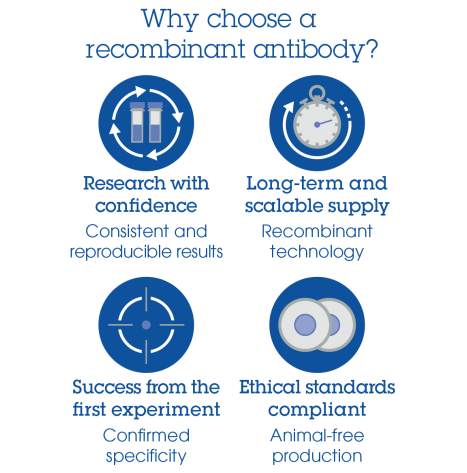


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] (ab256384)

Immunohistochemical analysis of paraffin-embedded human chondroblastoma tissue labeling Histone H3 (mutated K36 M) with ab256384 at 1/4000 dilution followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear staining in human chondroblastoma (PMID: 29757500). Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Goat Anti-Rabbit IgG H&L (HRP).

Heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0)



Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] (ab256384)

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