abcam

Product datasheet

Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade ab254401

Recombinant RabMAb

9 Images

Overview

Product name	Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade
Description	Rabbit monoclonal [EPR23520-5] to Histone H3.3 (mutated G34V) - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), IP, WB, ICC/IF, Dot blot, ChIP, IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK-293T transfected with Histone H3.3 G34V expression vector containing a myc-His-tag, whole cell lysate. IHC-P: Human giant tumor of bone.ICC/IF: 293T cells transfected with Histone H3.3 H3G34V-Myc plasmid.Flow Cyt (intra): 293T transfected myc-tagged Histone H3.3 H3G34V construct. IP: HEK-293T transfected with Histone H3.3 G34V expression vector containing a myc-His-tag whole cell lysate.
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: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR23520-5
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab254401 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)		1/500.
IP		Use at an assay dependent concentration.
WB		1/1000. Predicted molecular weight: 15 kDa.
ICC/IF		1/1000.
Dot blot		1/1000.
ChIP		Use 2 µg for 25 µg of chromatin.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Target

Function Variant histone H3 which replaces conventional H3 in a wide range of nucleosomes in active genes. Constitutes the predominant form of histone H3 in non-dividing cells and is incorporated into chromatin independently of DNA synthesis. Deposited at sites of nucleosomal displacement throughout transcribed genes, suggesting that it represents an epigenetic imprint of transcriptionally active chromatin. 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.

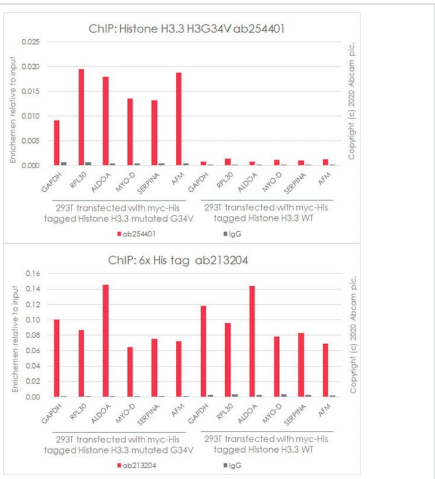
Developmental stage Expressed throughout the cell cycle independently of DNA synthesis.

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.
Specifically enriched in modifications associated with active chromatin such as methylation at Lys-5 (H3K4me), Lys-37 and Lys-80. 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), which are linked to gene repression, are underrepresented. 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. Phosphorylation on Ser-32 (H3S31ph) is specific to regions bordering centromeres in metaphase chromosomes.
Ubiquitinated. Monoubiquitinated by RAG1 in lymphoid cells, monoubiquitination is required for V(D)J recombination.

Cellular localization Nucleus. Chromosome.

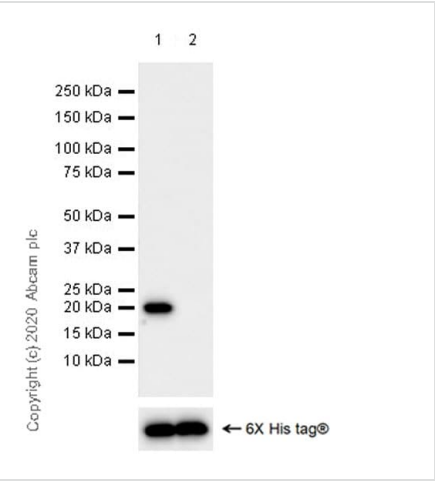
Images



ChIP - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade (ab254401)

Chromatin was prepared from HEK-293T transfected with myc-His tagged Histone H3.3 mutated G34V and Histone H3.3 WT cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with then formaldehyde for 10min.
The ChIP was performed with 25 µg of chromatin, 2 µg of ab254401 (red), or 2 µg of rabbit normal IgG ab172730 (gray) and 20 µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are located in the first kb of the transcribed region.

*https://www.abcam.com/resources?keywords=X%20ChIP%20protocol



Western blot - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade (ab254401)

All lanes : Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade (ab254401) at 1/1000 dilution

Lane 1 : HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34V expression vector containing a myc-His-tag®, whole cell lysate
Lane 2 : HEK-293T transfected with Histone H3.3 (WT) expression vector containing a myc-His-tag®, whole cell lysate

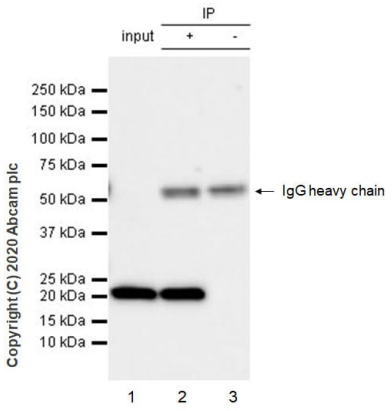
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (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: 10 seconds.



Immunoprecipitation - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade (ab254401)

Histone H3.3(mutated G34 V) was immunoprecipitated from 0.35 mg HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34V expression vector containing a myc-His-tag® whole cell lysate 10 µg with ab254401 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab254401 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/5000 dilution.

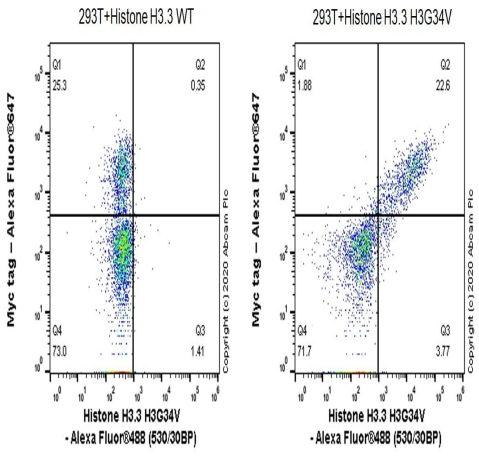
Lane 1: HEK-293T transfected with Histone H3.3 G34V expression vector containing a myc-His-tag® whole cell lysate 10 µg

Lane 2: ab254401 IP in HEK-293T transfected with Histone H3.3 G34V expression vector containing a myc-His-tag® whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab254401 in HEK-293T transfected with Histone H3.3 G34V expression vector containing a myc-His-tag® whole cell lysate

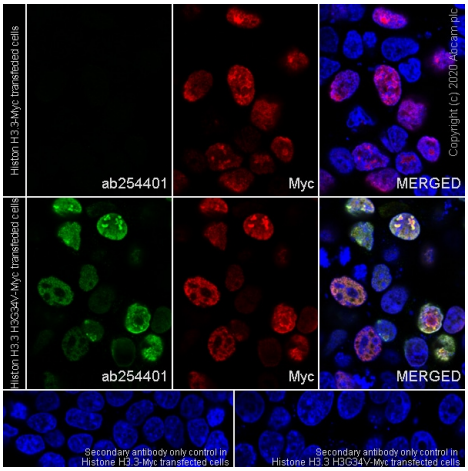
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 32 seconds.



Flow Cytometry (Intracellular) - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade (ab254401)

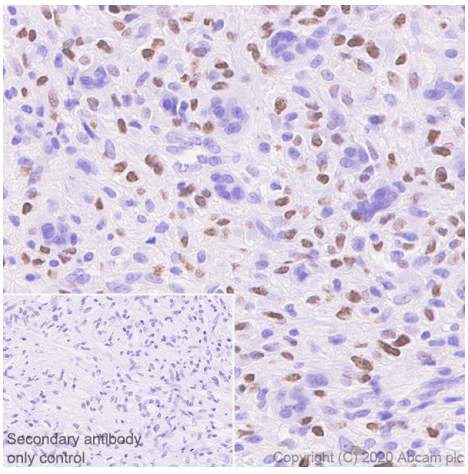
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HEK-293T (Human embryonic kidney epithelial cell) transfected with myc tagged Histone H3.3 WT construct (Left panel) and myc-tagged Histone H3.3 H3G34V construct (Right panel) cells labelling Histone H3.3 (mutated G34 V) with ab254401 at 1/500 dilution (0.1µg). A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade (ab254401)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T cells labelling Histone H3.3(mutated G34 V) with ab254401 at 1/1000 dilution, followed by ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 2 µg/ml dilution (Green). Confocal image showing nuclear staining in HEK-293T cells transfected with Histone H3.3 H3G34V-Myc plasmid, while no staining in HEK-293T cells transfected with H3.3 WT -Myc plasmid. Myc-Tag Mouse mAb (Alexa Fluor® 647) was used to counterstain tubulin at 1/200 dilution (Red). The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is ab150077 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1/1000 2 µg/ml dilution.

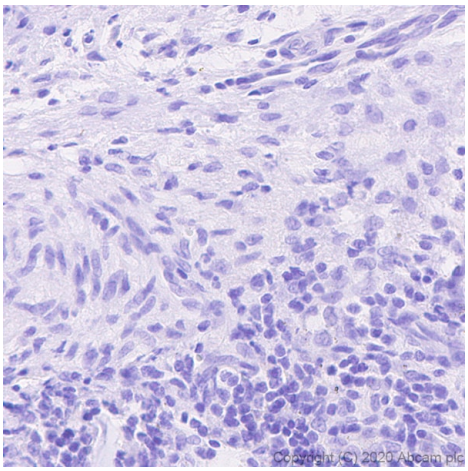


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade (ab254401)

Immunohistochemical analysis of paraffin-embedded human giant cell tumor of bone tissue labeling Histone H3.3(mutated G34 V) with ab254401 at 1/1000 dilution (0.542 µg/ml) dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human giant cell tumor of bone. (PMID: 29241742). The section was incubated with ab254401 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade (ab254401)

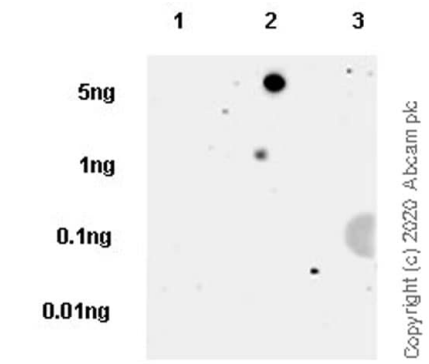
Immunohistochemical analysis of paraffin-embedded human chondroblastoma tissue labeling Histone H3.3(mutated G34 V) with ab254401 at 1/1000 dilution (0.542 µg/ml) dilution followed by ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Negative control: No staining on human chondroblastoma (PMID: 29241742).

The section was incubated with ab254401 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Dot Blot - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade (ab254401)

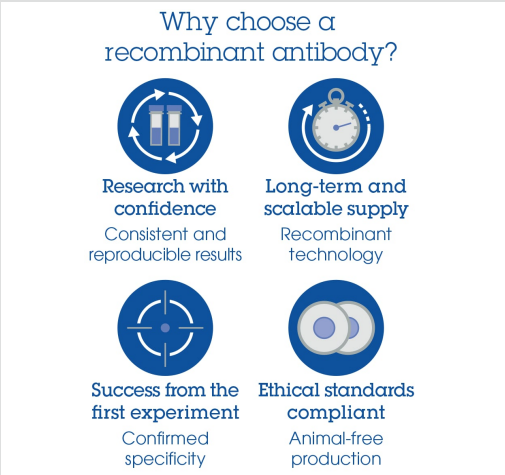
Dot blot analysis of Histone H3.3 (mutated G34 V) labeled with ab254401 at 1/1000 dilution.

Lane 1: Histone H3.3 H3G34V peptide (aa28-40).
Lane 2: Histone H3.3 H3G34V peptide (aa26-38).
Lane 3: Histone H3.3 WT peptide (aa26-40).

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

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.



Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade (ab254401)

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