

## Product datasheet

# Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade - BSA and Azide free ab272700

Recombinant RabMAb

7 Images

### Overview

<b>Product name</b>	Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade - BSA and Azide free
<b>Description</b>	Rabbit monoclonal [EPR23581-39] to Histone H3.3 (mutated G34W) - ChIP Grade – BSA and Azide free
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> Flow Cyt (Intra), ICC/IF, IHC-P, Dot blot, WB, ChIP
<b>Species reactivity</b>	<b>Reacts with:</b> Human
<b>Immunogen</b>	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	WB: HEK-293T transfected with Histone H3.3 G34W expression vector containing a myc-His-tag, whole cell lysate. IHC-P: Human giant tumor of bone. Flow Cyt (intra): 293T cells transfected with myc-tagged Histone H3.3 G34W construct. ICC/IF: 293T cells transfected with Histone H3.3 G34W-Myc plasmid.
<b>General notes</b>	<p>ab272700 is the carrier-free version of <a href="#">ab272691</a>.</p> <p>Our <b>carrier-free</b> 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 <b>conjugation kits</b> for antibody conjugates that are ready-to-use in as little as 20 minutes with &lt;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<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> 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"><li>- High batch-to-batch consistency and reproducibility</li><li>- Improved sensitivity and specificity</li><li>- Long-term security of supply</li><li>- Animal-free production</li></ul> <p>For more information <a href="#">see here</a>.</p>

Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to [RabMAb<sup>®</sup> patents](#).

## Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C. Do Not Freeze.
<b>Storage buffer</b>	pH: 7.2 Constituent: PBS
<b>Carrier free</b>	Yes
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR23581-39
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our [Abpromise guarantee](#) covers the use of ab272700 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)		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Dot blot		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 15 kDa.
ChIP		Use at an assay dependent concentration.

## 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 PAD4 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.

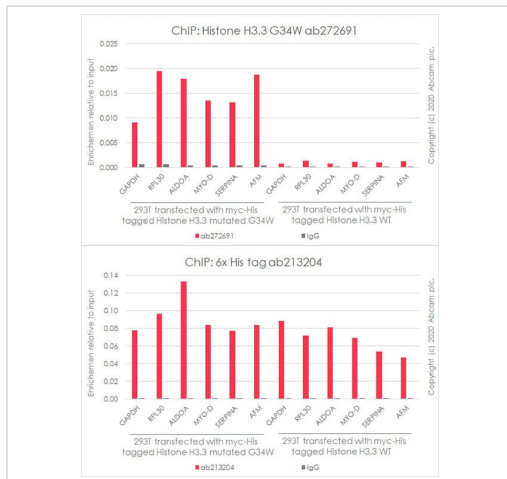
Phosphorylation at Ser-11 (H3S10ph), which is linked to gene activation, prevents methylation at Lys-10 (H3K9me) but facilitates acetylation of H3 and H4. Phosphorylation at Ser-11 (H3S10ph) by AURKB mediates the dissociation of HP1 proteins (CBX1, CBX3 and CBX5) from heterochromatin. Phosphorylation 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.



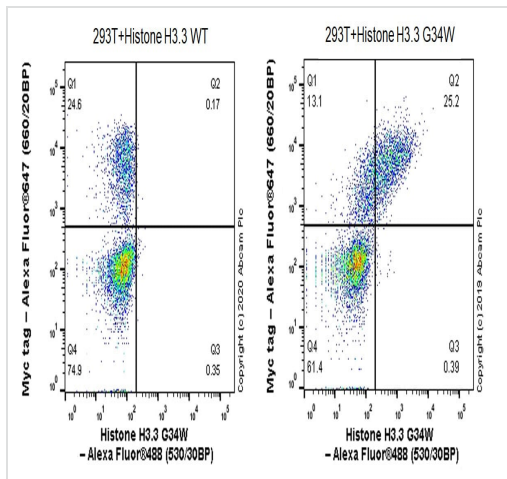
ChIP - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade - BSA and Azide free (ab272700)

Chromatin was prepared from 293T transfected with myc-His tagged Histone H3.3 mutated G34W and Histone H3.3 WT cells according to the Abcam Dual-X-ChIP protocol\*. Cells were fixed with formaldehyde for 10min.

The ChIP was performed with 25 µg of chromatin, 2 µg of **ab272691** (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>

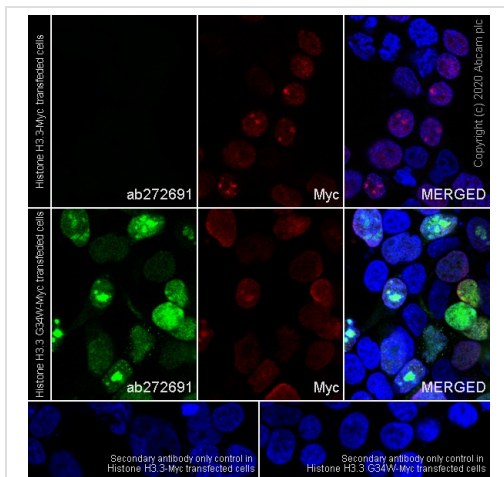
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab272691**).



Flow Cytometry (Intracellular) - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade - BSA and Azide free (ab272700)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed 90% methanol-permeabilized 293T (Human embryonic kidney epithelial cell) transfected with myc tagged Histone H3.3 WT construct (Left panel) and myc-tagged Histone H3.3 G34W construct (Right panel) cells labelling Histone H3.3 (mutated G34W) with **ab272691** at 1/50 dilution (1µg). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab272691**).

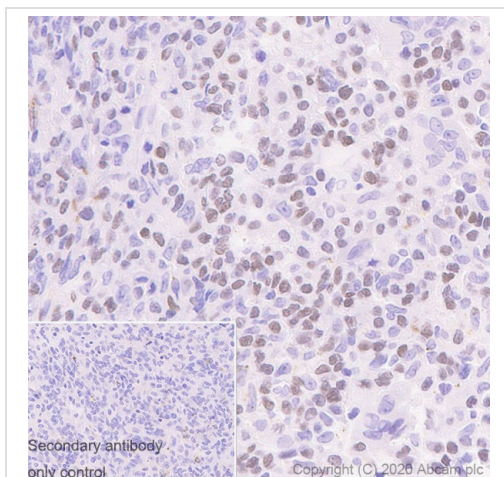


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade - BSA and Azide free (ab272700)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized 293T cells labelling Histone H3.3(mutated G34 W) with **ab272691** at 1/100 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 293T cells transfected with Histone H3.3 G34W-Myc plasmid, while no staining in 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab272691**).



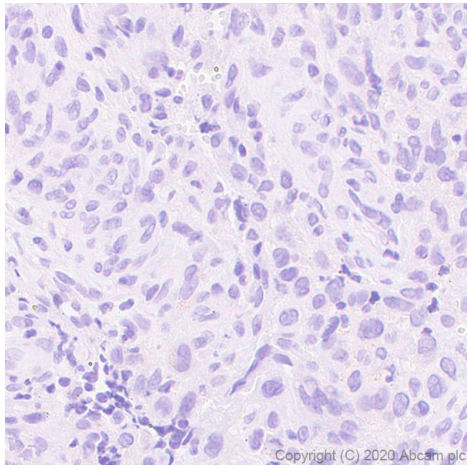
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade - BSA and Azide free (ab272700)

Immunohistochemical analysis of paraffin-embedded human giant cell tumor of bone tissue labeling Histone H3.3(mutated G34 W) with **ab272691** at 1/250 dilution followed by ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear staining in human giant cell tumor of bone (PMID: 29757500). Counterstained with Hematoxylin.

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

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab272691**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade - BSA and Azide free (ab272700)

Immunohistochemical analysis of paraffin-embedded human chondroblastoma tissue labeling Histone H3.3(mutated G34 W) with **ab272691** at 1/250 dilution followed by ready to use Goat Anti-Rabbit IgG H&L (HRP).

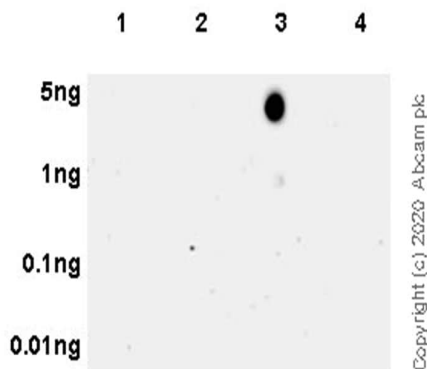
**Negative control:** No staining in human chondroblastoma (PMID: 29757500).

Counterstained with Hematoxylin.

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

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab272691**).



Dot Blot - Anti-Histone H3.3 (mutated G34W) antibody [EPR23581-39] - ChIP Grade - BSA and Azide free (ab272700)

Dot blot analysis of Histone H3.3 (mutated G34 W) labeled with **ab272691** at 1/1000 dilution.

**Lane 1:** Histone H3.3 H3G34W peptide (aa28-37).

**Lane 2:** Histone H3.3 H3G34W peptide (aa33-43).

**Lane 3:** Histone H3.3 H3G34W peptide (aa28-43).

**Lane 4:** Histone H3.3 WT peptide (aa28-43).

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab272691**).

### 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 H3.3 (mutated G34W) antibody  
[EPR23581-39] - ChIP Grade - BSA and Azide free  
(ab272700)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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