abcam

ab254401

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

Recombinant | RabMAb | 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
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR23520-5
Purification technique	Affinity purification Protein A
Concentration	0.519 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Transfected cell lysate
Dilution info	-
Notes	-

Expected

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

Not recommended

Species	Transfected cell line, Synthetic peptide, Transfected cell lysate - Human, Mouse, Rat
Dilution info	-
Notes	-

ChIP

Tested

Species	Transfected cell line
Dilution info	2 µg for 25 µg chromatin
Notes	-

Expected

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

Not recommended

Species	Transfected cell lysate, Synthetic peptide, Transfected cell lysate - Human, Mouse, Rat
Dilution info	-
Notes	-

Dot

Tested

Species	Synthetic peptide
Dilution info	1/1000
Notes	-

Expected

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

Not recommended

Species	Transfected cell lysate, Transfected cell line, Transfected cell lysate - Human, Mouse, Rat
Dilution info	-
Notes	-

WB

Tested

Species	Transfected cell lysate - Human
Dilution info	1/1000
Notes	-

Expected

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

Not recommended

Species	Transfected cell lysate, Transfected cell line, Synthetic peptide, Mouse, Rat
Dilution info	-

Notes	-

ICC/IF

Tested

Species	Transfected cell line
Dilution info	1/1000
Notes	-

Expected

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

Not recommended

Species	Transfected cell lysate, Synthetic peptide, Transfected cell lysate - Human, Mouse, Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Transfected cell line
Dilution info	1/500
Notes	-

Expected

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

Not recommended

Species	Transfected cell lysate, Synthetic peptide, Transfected cell lysate - Human, Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Mouse, Rat, Transfected cell lysate, Transfected cell line, Synthetic peptide, Transfected cell lysate - Human
Dilution info	-
Notes	-

Target data

See full target information H3-3A G34V

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.

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

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

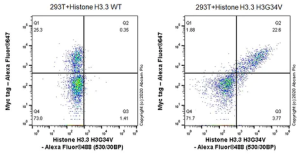
We do not recommend this combination. It is not covered by our product promise.

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.

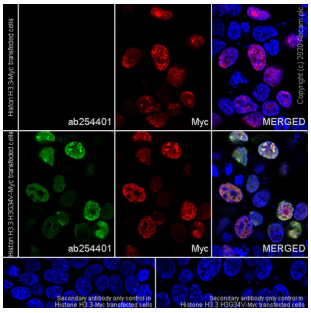
8 product images



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

Histone H3.3 (mutated G34V) Flow Cytometry (Intracellular) staining using rabbit Anti-Histone H3.3 (mutated G34V) antibody

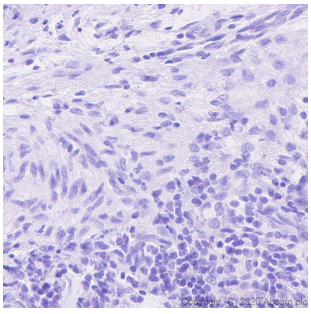
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized HEK-293T (Human embryonic kidney epithelial cell) transfected with myc tagged Histone H3.3 WT construct (Left panel) and myc-tagged Histone H3.3 H3G34V construct (Right panel) cells labelling Histone H3.3 (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)

Histone H3.3 (mutated G34V) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Histone H3.3 (mutated G34V) antibody

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)

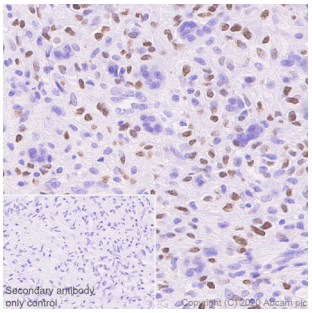
Histone H3.3 (mutated G34V) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Histone H3.3 (mutated G34V) antibody

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.

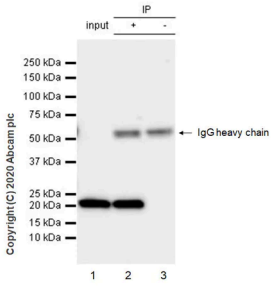


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade (ab254401)

Histone H3.3 (mutated G34V) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Histone H3.3 (mutated G34V) antibody

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.



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

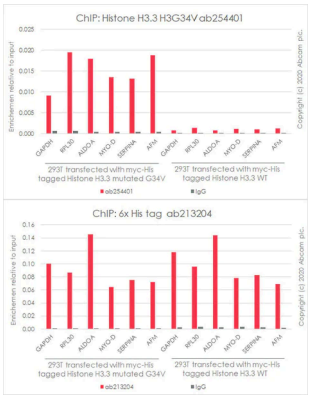
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab254401 in HEK-293T transfected with Histone H3.3 G34V expression vector containing a myc-His-tag® whole cell lysate

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

Exposure time: 32 seconds.

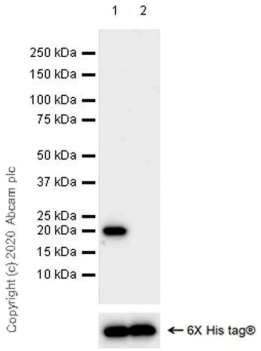
All lanes:
Immunoprecipitation - Anti-Histone H3.3 (mutated G34V) antibody [EPR23520-5] - ChIP Grade (ab254401)

Predicted band size: 15 kDa



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.



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

Histone H3.3 (mutated G34V) Western blot staining using rabbit Anti-Histone H3.3 (mutated G34V) antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
Exposure time: 10 seconds.

All lanes:
Western blot - 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 at 20 µg

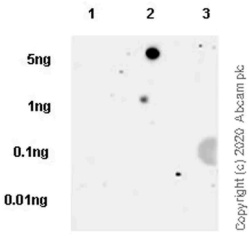
Lane 2:
HEK-293T transfected with Histone H3.3 (WT) expression vector containing a myc-His-tag®, whole cell lysate at 20 µg

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



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

Histone H3.3 (mutated G34V) Dot Blot staining using rabbit Anti-Histone H3.3 (mutated G34V) antibody

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.

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