abcam

ab273635

Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade - BSA and Azide free

Recombinant RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR23519-91
Purification technique	Affinity purification Protein A
Concentration	1.045 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-

Notes	-

Not recommended

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

ICC/IF

Not recommended

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

IP

Tested

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

Expected

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

Not recommended

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

ChIP

Tested

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

Expected

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

Not recommended

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

Dot

Tested

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Expected

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

Not recommended

Species	Transfected cell lysate - Human, Transfected cell line - Human

Dilution info	-
Notes	-

WB

Tested

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

Expected

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

Not recommended

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

Flow Cyt (Intra)

Tested

Species	Transfected cell line - Human
Dilution info	-
Notes	-

Expected

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

Not recommended

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

Target data

See full target information H3-3A G34R

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	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab273635 is the carrier-free version of ab254402.

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.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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

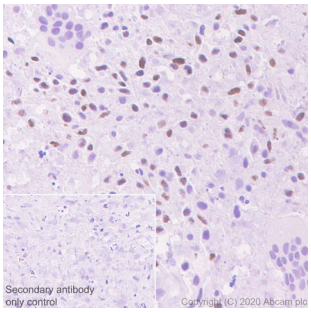
We do not recommend this combination. It is not covered by our product promise.

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.

7 product images



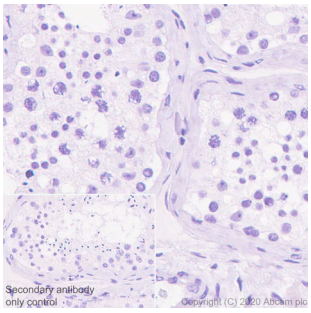
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade - BSA and Azide free (ab273635)

Histone H3.3 (mutated G34R) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Histone H3.3 (mutated G34R) antibody

Immunohistochemical analysis of paraffin-embedded Human giant cell tumor of bone tissue labeling Histone H3.3 (mutated G34 R) with ab254402 at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining on human giant cell tumor of bone (PMID: 29241742). The section was incubated with ab254402 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 a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Citrate buffer (pH 6.0, epitope retrieval solution 1) for 20 mins

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab254402).



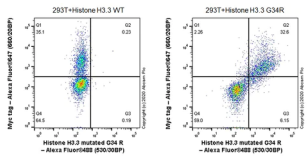
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade - BSA and Azide free (ab273635)

Histone H3.3 (mutated G34R) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Histone H3.3 (mutated G34R) antibody

Immunohistochemical analysis of paraffin-embedded Human testis tissue labeling Histone H3.3 (mutated G34 R) with ab254402 at 1/2000 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). **Negative control:** no staining on human testis. The section was incubated with ab254402 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 a 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab254402).

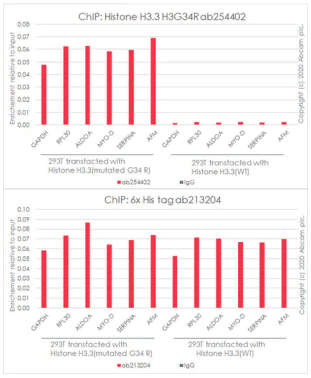


Flow Cytometry (Intracellular) - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade - BSA and Azide free (ab273635)

Histone H3.3 (mutated G34R) Flow Cytometry (Intracellular) staining using rabbit Anti-Histone H3.3 (mutated G34R) antibody

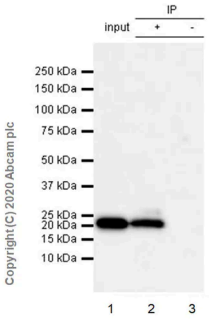
Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized HEK-293T (Human embryonic kidney epithelial cell) (transfected with myc-tagged Histone H3.3 WT expression vector) (Left) or myc-tagged Histone H3.3 G34R expression vector (Right) cells labelling

Histone H3.3 (mutated G34 R) with ab254402 at 1/5000 dilution (0.01ug) (Both panels). 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 (ab254402).



ChIP - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade - BSA and Azide free (ab273635)

Chromatin was prepared from 293T transfacted with Histone H3.3(mutated G34 R) and 293T transfacted with Histone H3.3(WT)
cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25 µg of chromatin, 2 µg of ab254402 (red), or 2 µg of rabbit normal IgG ab172730 (gray) and 25 µl of Protein A/G Dynabeads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab254402).



Immunoprecipitation - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade - BSA and Azide free (ab273635)

Histone H3.3 (mutated G34 R) was immunoprecipitated from 0.35 mg HEK-293T (human embryonic kidney) (transfected with Histone H3.3 G34R expression vector containing a myc-His-tag®) whole cell lysate with ab254402 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab254402 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.
Lane 1: HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34R expression vector containing a myc-His-tag®, whole cell lysate 10 ug

Lane 2: ab254402 IP in HEK-293T transfected with Histone H3.3 G34R expression vector containing a myc-His-tag® whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab254402 in HEK-293T transfected with Histone H3.3 G34R expression vector containing a myc-His-tag® whole cell lysate

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

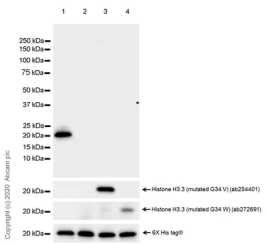
Exposure time: 41 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab254402).

All lanes:
Immunoprecipitation - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade (ab254402)

Predicted band size: 15 kDa

Observed band size: 20 kDa



Western blot - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade - BSA and Azide free (ab273635)

Histone H3.3 (mutated G34R) Western blot staining using rabbit Anti-Histone H3.3 (mutated G34R) antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
Exposure time: 26 seconds .

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab254402).

All lanes:
Western blot - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade (ab254402) at 1/1000 dilution

Lane 1:
HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34R 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

Lane 3:
HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34V expression vector containing a myc-His-tag®, whole cell lysate at 20 µg

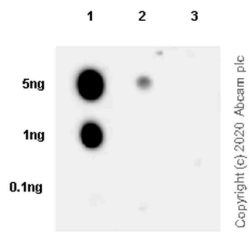
Lane 4:
HEK-293T (human embryonic kidney) transfected with Histone H3.3 G34W expression vector containing a myc-His-tag®, whole cell lysate at 20 µg

Secondary

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

Predicted band size: 15 kDa

Observed band size: 20 kDa



Dot Blot - Anti-Histone H3.3 (mutated G34R) antibody [EPR23519-91] - ChIP Grade - BSA and Azide free (ab273635)

Histone H3.3 (mutated G34R) Dot Blot staining using rabbit Anti-Histone H3.3 (mutated G34R) antibody

Dot blot analysis of Histone H3.3 (mutated G34 R) labeled with ab254402 at 1/1000 dilution.
Lane 1: Histone H3.3 H3G34R peptide (aa28-40).
Lane 2: Histone H3.3 H3G34R peptide (aa26-36).
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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab254402).

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