abcam

ab256384

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

Recombinant | RabMAb | Advanced Validation

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	EPR23614-91
Purification technique	Affinity purification Protein A
Concentration	0.493 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ChIC/CUT&RUN-seq

Tested

Species	Transfected cell line - Human
Dilution info	3 µg
Notes	-

Expected

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

Not recommended

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

IP

Tested

Species	Transfected cell lysate - Human
Dilution info	1/30
Notes	-

Expected

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

Not recommended

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

IHC-P

Tested

Species	Human
Dilution info	1/4000
Notes	Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0)

Not recommended

Species	Mouse
Dilution info	-
Notes	Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0)

Species	Rat
Dilution info	-
Notes	Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0)

Species	Transfected cell line - Human
Dilution info	-

Notes	-

Species	Transfected cell lysate - Human
Dilution info	-
Notes	-

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

I-ELISA

Tested

Species	Synthetic peptide - Human
Dilution info	1 µg/mL
Notes	-

Expected

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

Not recommended

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

ICC/IF

Tested

Species	Human
Dilution info	1/50

Notes	-

Not recommended

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

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

Not recommended

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

ChIP

Not recommended

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

Target data

See full target information H3-3A K36M

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

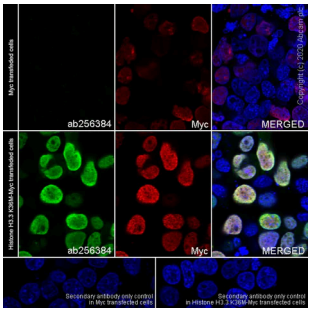
We do not recommend this combination. It is not covered by our product promise.

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.

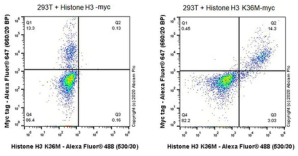
9 product images



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

Histone H3 (mutated K36M) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Histone H3 (mutated K36M) antibody

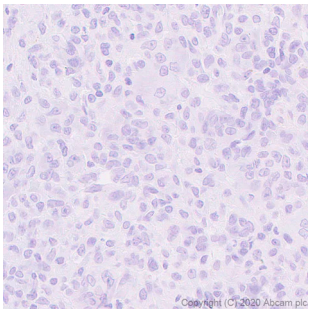
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293 cells labelling Histone H3 (mutated K36 M) with ab256384 at 1/50 dilution, followed by a secondary antibody at 1/1000 dilution (Green). Confocal image showing nuclear staining in HEK-293 cell line transfected 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).



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

Histone H3 (mutated K36M) Flow Cytometry (Intracellular) staining using rabbit Anti-Histone H3 (mutated K36M) antibody

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.

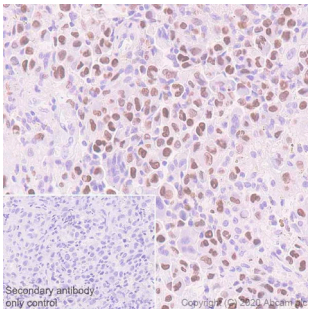


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] (ab256384)

Histone H3 (mutated K36M) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Histone H3 (mutated K36M) antibody

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)

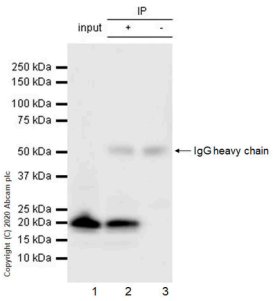


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] (ab256384)

Histone H3 (mutated K36M) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Histone H3 (mutated K36M) antibody

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)



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

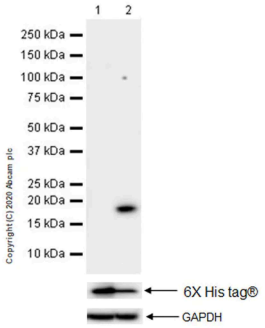
Lane 3:Rabbit monoclonal IgG (ab172730) instead of ab256384 in HEK-293 transfected with Histone H3.3 K36M (mutate) expression vector containing a myc-His-tag®, whole cell lysate

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

Exposure time: 10 seconds

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

Predicted band size: 15 kDa



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

Histone H3 (mutated K36M) Western blot staining using rabbit Anti-Histone H3 (mutated K36M) antibody

Blocking/Diluting buffer and concentration: 5% NFDM/TBST
All lanes:
Western blot - 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 at 20 µg

Lane 2:
HEK-293 transfected with Histone H3.1 K36M (mutant) expression vector containing a myc-His-tag®, whole cell lysate at 20 µg

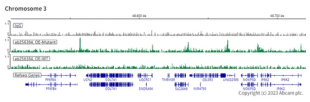
Secondary

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

Predicted band size: 15 kDa

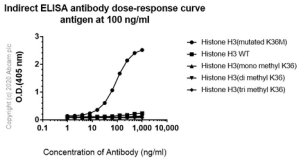
Observed band size: 19 kDa

Exposure time: 62s



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 found in the Protocol booklet in the Product Protocol section. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

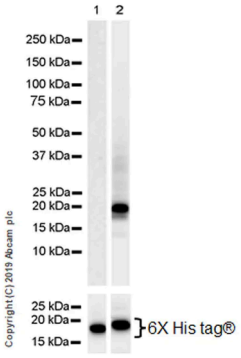


Indirect ELISA - Anti-Histone H3 (mutated K36M) antibody [EPR23614-91] (ab256384)

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.



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

Histone H3 (mutated K36M) Western blot staining using rabbit Anti-Histone H3 (mutated K36M) antibody

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

All lanes:
Western blot - 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 at 10 µg

Lane 2:
HEK-293 transfected with Histone H3.3 K36M (mutate) expression vector containing a myc-His-tag®, whole cell lysate at 10 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

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