abcam

Product datasheet

Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] - ChIP Grade ab282572

Recombinant RabMAb

1 References 12 Images

Overview

Product name	Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] - ChIP Grade
Description	Rabbit monoclonal [EPR23525-232] to Histone H3 (tri methyl K36) - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** ChIP-sequencing, WB, IHC-P, Flow Cyt (Intra), PepArr, ICC/IF **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, Saos-2, C2C12 and NIH/3T3, C6 lysates. IHC-P: Human colon, Human colon carcinoma and Mouse lung, Rat lung tissues. ICC/IF: HeLa, NIH/3T3 cells. Flow Cyt (Intra): HeLa, NIH/3T3 cells. ChIP-sequencing: HeLa cells.
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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59.94% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR23525-232
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab282572 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
ChIP-sequencing		Use at an assay dependent concentration.
WB		1/1000. Predicted molecular weight: 15 kDa.
IHC-P		1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/500.
PepArr		Use a concentration of 0.05 µg/ml.
ICC/IF		1/500.

Application notes Is unsuitable for IP.

Target

Function Core component of nucleosome. 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.

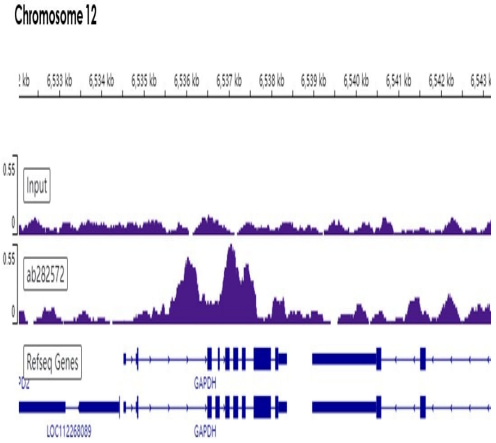
Developmental stage Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

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.
Methylation at Lys-5 (H3K4me), Lys-37 (H3K36me) and Lys-80 (H3K79me) are linked to gene activation. 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) are linked to gene repression. 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.
Monoubiquitinated by RAG1 in lymphoid cells, monoubiquitination is required for V(D)J recombination (By similarity). Ubiquitinated by the CUL4-DDB-RBX1 complex in response to ultraviolet irradiation. This may weaken the interaction between histones and DNA and facilitate DNA accessibility to repair proteins.

Cellular localization Nucleus. Chromosome.

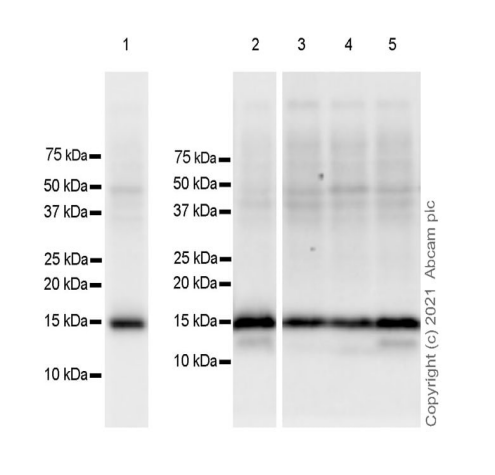
Images



ChIP-sequencing - Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] (ab282572)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 HeLa cells and 4 µg ab282572 [EPR23525-232]. ChIP DNA was sequenced on the Illumnia NovaSeqn 6000 to a depth of 30 million reads.

Additional screenshots and mapped reads can be downloaded **here**.



Western blot - Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] (ab282572)

All lanes : Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] - ChIP Grade (ab282572) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : Saos-2 (human osteosarcoma epithelial) whole cell lysate
Lane 3 : C2C12 (mouse myoblasts myoblast) whole cell lysate
Lane 4 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate
Lane 5 : C6 (rat glial tumor glial cell) whole cell lysate

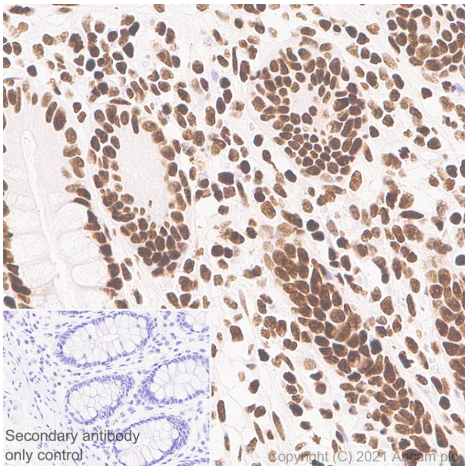
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 15 kDa
Observed band size: 15 kDa

Blocking and diluting buffer and concentration: 5% BSA/TBST

Exposure time: Lanes 1-2: 15 seconds;Lanes 3-5: 26 seconds.

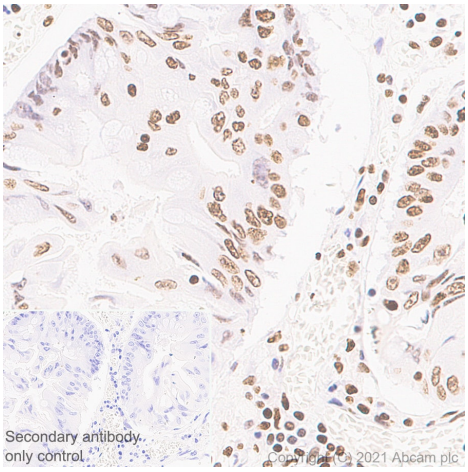


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] (ab282572)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labelling Histone H3 (tri methyl K36) with ab282572 at 1/500 (0.964 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining in human colon. The section was incubated with ab282572 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

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

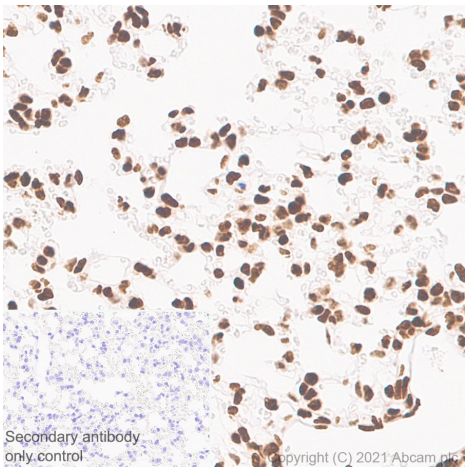


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] (ab282572)

Immunohistochemical analysis of paraffin-embedded Human colon carcinoma tissue labelling Histone H3 (tri methyl K36) with ab282572 at 1/500 (0.964 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining in human colon carcinoma. The section was incubated with ab282572 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

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

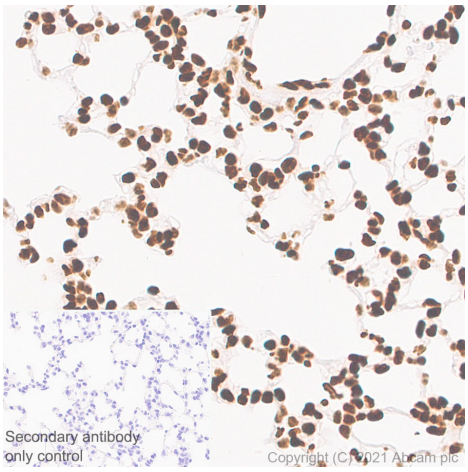


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] (ab282572)

Immunohistochemical analysis of paraffin-embedded Mouse lung tissue labelling Histone H3 (tri methyl K36) with ab282572 at 1/500 (0.964 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining in mouse lung. The section was incubated with ab282572 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

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

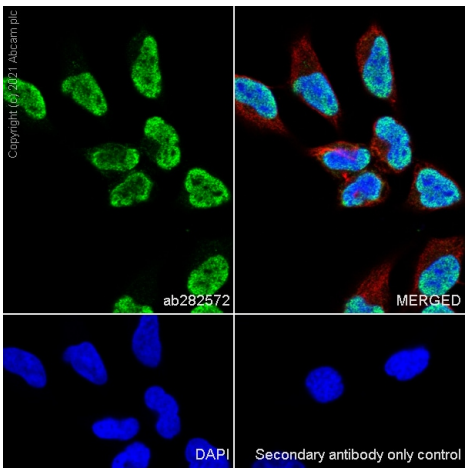


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] (ab282572)

Immunohistochemical analysis of paraffin-embedded Rat lung tissue labelling Histone H3 (tri methyl K36) with ab282572 at 1/500 (0.964 ug/ml) followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Nuclear staining in rat lung. The section was incubated with ab282572 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 LeicaDS9800 (Bond™ Polymer Refine Detection).

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

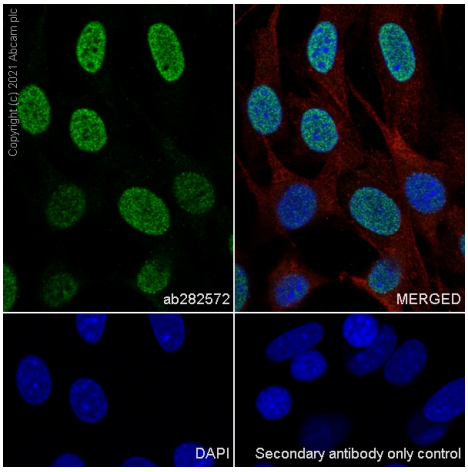


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] - ChIP Grade (ab282572)

Immunofluorescent analysis of 100% methanol-fixed, 0.1% TritonX-100 permeabilized HeLa cells labelling Histone H3 (tri methyl K36) with ab282572 at 1/500 (0.964 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing nuclear staining in HeLa cell line is observed.

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.

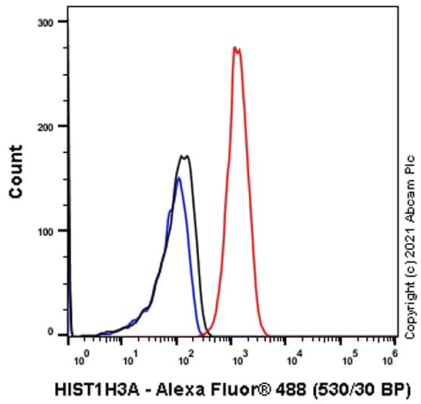


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] - ChIP Grade (ab282572)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized NIH/3T3 cells labelling Histone H3 (tri methyl K36) with ab282572 at 1/500 (0.964 ug/ml) dilution, followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing nuclear staining in NIH/3T3 cell line is observed.

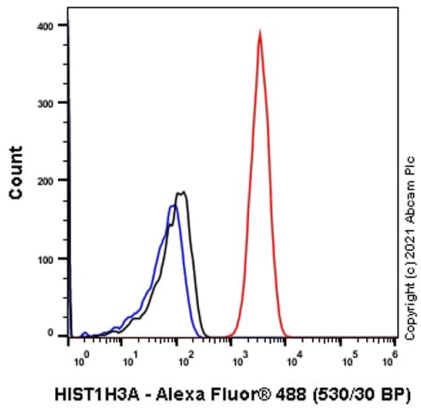
ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.



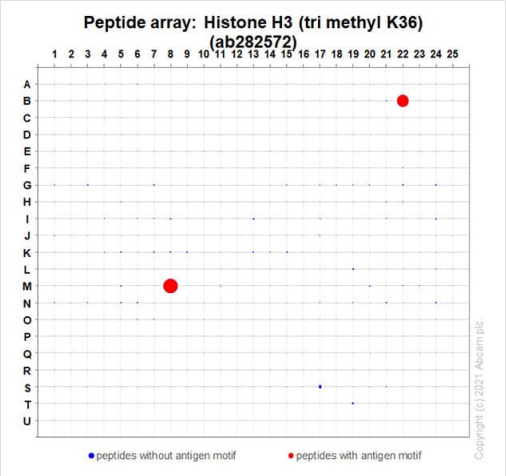
Flow Cytometry (Intracellular) - Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] - ChIP Grade (ab282572)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling Histone H3 (tri methyl K36) with ab282572 at 1/500 dilution (0.1ug)/ (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) 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 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] - ChIP Grade (ab282572)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized NIH/3T3 (Mouse embryonic fibroblast) cells labelling Histone H3 (tri methyl K36) with ab282572 at 1/500 dilution (0.1ug)/ (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) 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 dilution was used as the secondary antibody.



Peptide Array - Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] (ab282572)

Peptide array analysis of ab282572 at (0.05ug/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Fluo 647nm conjugated at 1:50,000 dilution.

All batches of **ab253206** are tested in Peptide Array against 501 different modified and unmodified histone peptides; each peptide is printed on the array at six concentrations (each in triplicate).
Circle area represents affinity between the antibody and a peptide: all antigen-containing peptides are displayed as red circles, all other peptides as blue circles. The affinity is calculated as the area under the curve when antibody binding values are plotted against the corresponding peptide concentration. Each circle area is normalized to the peptide with the strongest affinity.

Anti-Histone H3 (tri methyl K36) antibody [EPR23525-232] (ab282572)

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

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