abcam

Product datasheet

Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade ab32356

Recombinant RabMAb

9 Abreviews 117 References 11 Images

Overview

Product name	Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade
Description	Rabbit monoclonal [Y47] to Histone H3 (di methyl K4) - ChIP Grade
Host species	Rabbit
Specificity	This antibody only detects Histone H3 dimethylated on Lysine 4.
Tested applications	**Suitable for:** CHIPseq, IHC-Fr, ChIP, WB, IHC-P, ICC/IF, IP, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Chicken, Cow, Human, Monkey, Rice **Predicted to work with:** Sheep, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Zebrafish, Mammals
Immunogen	Synthetic peptide within Human Histone H3 aa 1-100 (di methyl K4). The exact sequence is proprietary.
Positive control	WB: HeLa, HEK-293, SH-SY5Y, C6 L6, NIH/3T3, COS-1, UMNSAH/DF-1 and MDBK cell lysates. ICC/IF: HepG2 cells. Flow Cyt: HeLa cells. IHC-P: Human cervical carcinoma, mouse colon and rat spleen tissues.
General notes	A trial size is available to purchase for this antibody. Every new batch of this antibody is tested in house in ChIP. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMab® patents. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.** This product is a recombinant rabbit monoclonal antibody.

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: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	Y47
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab32356** 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
ChIPseq		Use at an assay dependent concentration. PubMed: 19581485
IHC-Fr	★★★★★	Use at an assay dependent concentration.
ChIP	★★★★★	Use 2 µg for 25 µg of chromatin.
WB	★★★★★	1/2000. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).
IHC-P		1/800.
ICC/IF	★★★★★	1/1000.
IP		Use at an assay dependent concentration. PubMed: 22086061
Flow Cyt		1/20 - 1/100. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

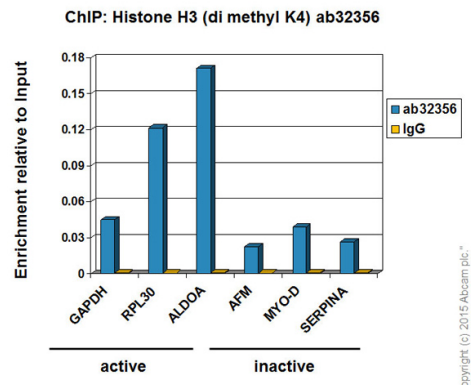
Target

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.

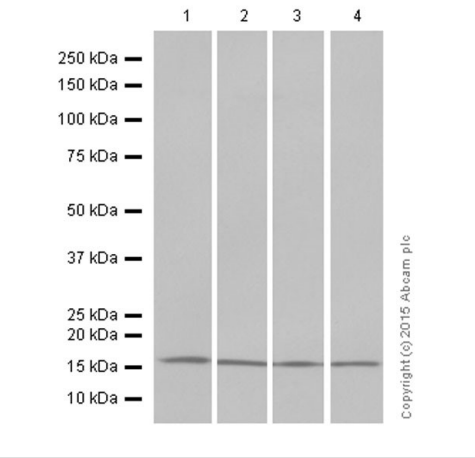
Cellular localization Nucleus. Chromosome.

Images



ChIP - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of ab32356 (blue), and 20µl of Anti Rabbit IgG sepharose beads. 2µg of rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach). Primers and probes are located in the first kb of the transcribed region.



Western blot - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

All lanes : Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356) at 1/10000 dilution (purified)

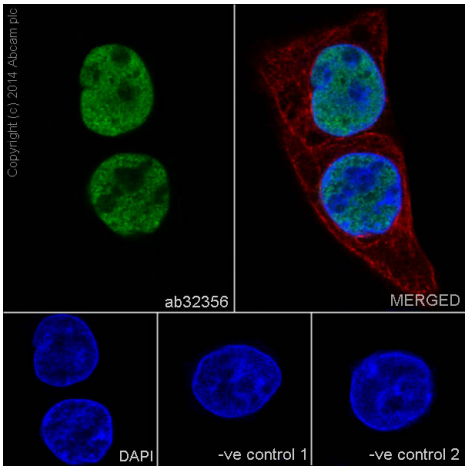
Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 3 : SH-SY5Y (Human neuroblastoma cell line from bone marrow) whole cell lysate
Lane 4 : C6 (Rat glial tumor cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 15 kDa
Additional bands at: 17 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 5 seconds

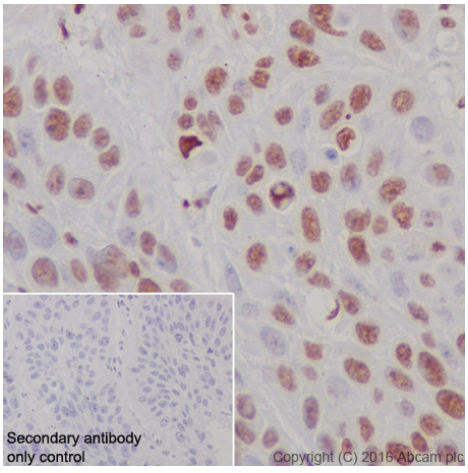


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Immunocytochemistry/Immunofluorescence analysis of HepG2 cells labelling Histone H3 (di methyl K4) with purified ab32356 at a dilution of 1/1000. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. The cells were co-stained with ab7291, a mouse anti-tubulin (1/1000) using ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) as the secondary antibody. Nuclei counterstained with DAPI (blue).

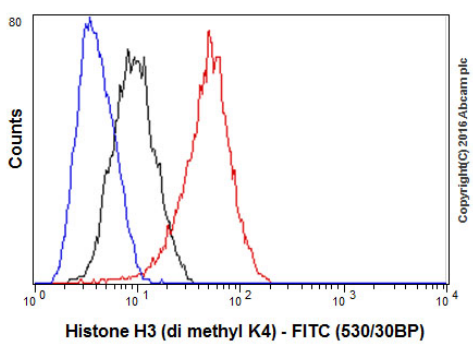
Control 1: primary antibody (1/1000) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).



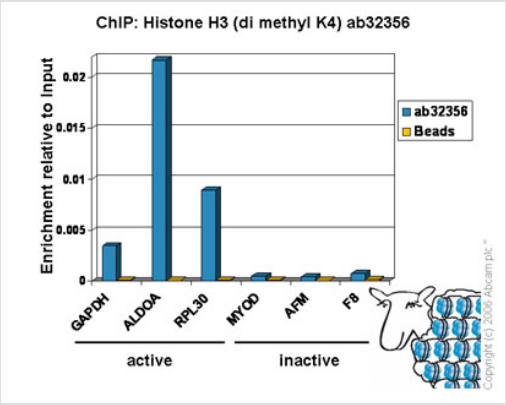
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labelling Histone H3 (di methyl K4) with purified ab32356 at a dilution of 1/800. Antigen retrieval was performed using Tris/EDTA buffer, pH9. ab97051, a HRP-conjugated goat anti-rabbit IgG H&L was used as the secondary antibody (1/500). Counter stained with hematoxylin.



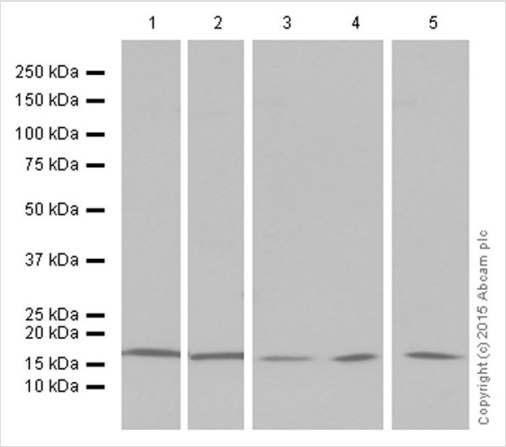
Flow Cytometry - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Flow Cytometry analysis of HeLa cells labelling Histone H3 (di methyl K4) with purified ab32356 at 1/150 (red). Cells were fixed with 80% methanol. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG (ab172730). Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



ChIP - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Chromatin was prepared from Hela cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25µg of chromatin, 8µl of ab32356 (blue), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach). Primers and probes are located in the first kb of the transcribed region. Every new batch of this antibody is tested at Abcam in ChIP.



Western blot - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

All lanes : Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356) at 1/2000 dilution (purified)

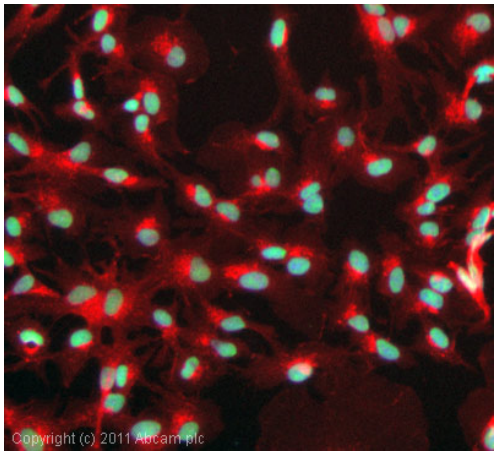
Lane 1 : L6 (Rat skeletal muscle cell line) whole cell lysate
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate
Lane 3 : COS-1 (African green monkey kidney fibroblast-like cell line) whole cell lysate
Lane 4 : UMNSAH/DF-1 (Transformed chicken embryonic fibroblast cell line) whole cell lysate
Lane 5 : MDBK (Bovine kidney cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution

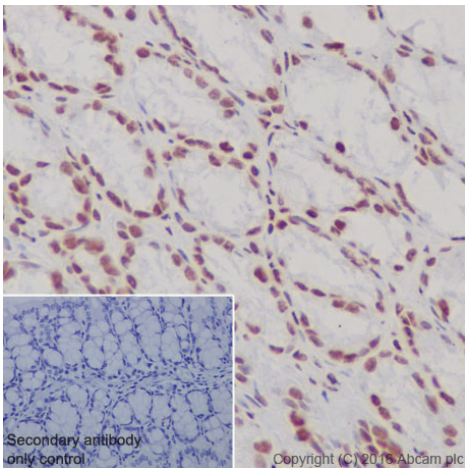
Predicted band size: 15 kDa
Observed band size: 17 kDa
why is the actual band size different from the predicted?

Exposure time: 5 seconds



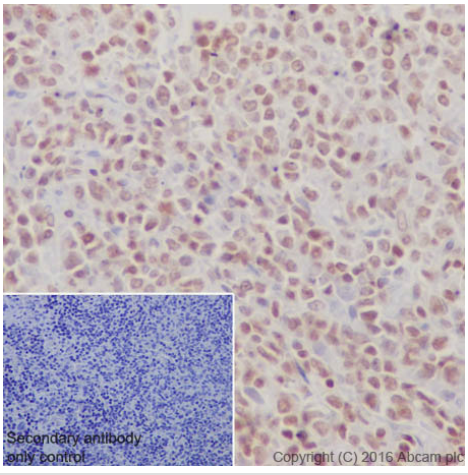
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

ICC/IF image of unpurified ab32356 stained HepG2 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab32356 , 5µg/ml) overnight at +4°C. The secondary antibody (green) was a goat anti-rabbit DyLight® 488 (IgG - H&L, pre-adsorbed) (ab96899) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



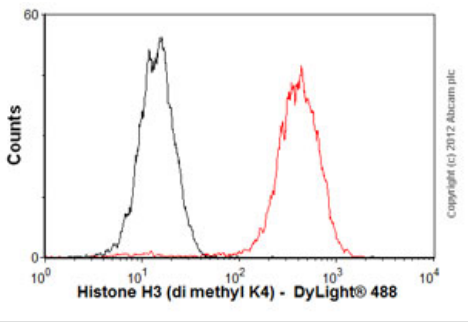
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse colon tissue labelling Histone H3 (di methyl K4) with purified ab32356 at a dilution of 1/800. Antigen retrieval was performed using Tris/EDTA buffer, pH9. ab97051, a HRP-conjugated goat anti-rabbit IgG H&L was used as the secondary antibody (1/500). Counter stained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat spleen tissue labelling Histone H3 (di methyl K4) with purified ab32356 at a dilution of 1/800. Antigen retrieval was performed using Tris/EDTA buffer, pH9. ab97051, a HRP-conjugated goat anti-rabbit IgG H&L was used as the secondary antibody (1/500). Counter stained with hematoxylin.



Flow Cytometry - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Overlay histogram showing HeLa cells stained with unpurified ab32356 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32356, 1/100 dilution) for 30 min at 22ºC. The secondary antibody used was a goat anti-rabbit DyLight® 488 (IgG; H+L) (ab96899) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

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