abcam

Product datasheet

Anti-Histone H3 (di methyl K27, tri methyl K27) antibody [mAbcam 6147] ab6147

9 Abreviews 31 References 7 图像

概述

产品名称	Anti-Histone H3 (di methyl K27, tri methyl K27)**抗体**[mAbcam 6147]
描述	**小鼠单克隆抗体**[mAbcam 6147] to Histone H3 (di methyl K27, tri methyl K27)
宿主	Mouse
特异性	By Western blot, this antibody is blocked strongly by di and tri methyl K27 peptides and does not detect a band in Eed KO mouse ES cell lysates (which lack both di and tri methyl K27). All batches of ab6147 have >40% cross reactivity with both H3K27me2 and H3K27me3 as shown by ELISA. The sequence which it reacts with is found in all Mammals and a wide range of other species, including D. melanogaster, Arabidopsis, Chicken and Xenopus. The antibody will react with any of the above species where the modification is present. Reactivity is not certain in S. pombe and S. cerevisiae as the equivalent protein sequence differs slightly from species listed above.
经测试应用	**适用于:** ICC/IF, ELISA, WB, Flow Cyt (Intra)
种属反应性	**与反应:** Mouse, Cow, Human **预测可用于:** Rat, Chicken, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster **不与反应:** Carp
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab1782**)
阳性对照	Human Lung
常规说明	Hybridomas were prepared and the resulting clones were positively screened by ELISA against the immunising peptide. Clones were negatively screened against both the corresponding unmodified peptide and also against a peptide corresponding to tri methylated K9 of Histone H3. This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

性能

形式	Liquid
存放说明	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -80°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS
纯度	Protein G purified
克隆	单克隆
克隆编号	mAbcam 6147
同种型	IgG1
轻链类型	kappa

应用

The Abpromise guarantee Abpromise™承诺保证使用ab6147于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF	★★★★★ (4)	Use a concentration of 1 - 5 µg/ml.
ELISA		Use a concentration of 0.025 - 1 µg/ml.
WB	★★★★☆ (3)	Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).Can be blocked with **Human Histone H3 (tri methyl K27) peptide (ab1782)**.
Flow Cyt (Intra)		Use 1-2µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

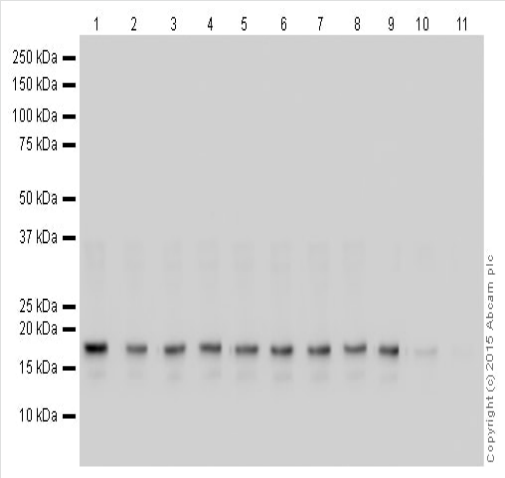
靶标

功能	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.
序列相似性	Belongs to the histone H3 family.
发展阶段	Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.
翻译后修饰	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.

细胞定位 Nucleus. Chromosome.

图片



Western blot - Anti-Histone H3 (di methyl K27, tri methyl K27) antibody [mAbcam 6147] (ab6147)

All lanes : Anti-Histone H3 (di methyl K27, tri methyl K27) antibody [mAbcam 6147] (ab6147) at 1 µg/ml

Lane 1 : Calf Thymus Histone Preparation Nuclear Lysate
Lane 2 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (unmodified) peptide (**ab2623**) at 1 µg
Lane 3 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K4) peptide (**ab1340**) at 1 µg
Lane 4 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K4) peptide (**ab7768**) at 1 µg
Lane 5 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K4) peptide (**ab1342**) at 1 µg
Lane 6 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K9) peptide (**ab1771**) at 1 µg
Lane 7 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K9) peptide (**ab1772**) at 1 µg
Lane 8 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K9) peptide (**ab1773**) at 1 µg
Lane 9 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K27) peptide (**ab1780**) at 1 µg
Lane 10 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K27) peptide (**ab1781**) at 1 µg
Lane 11 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K27) peptide (**ab1782**) at 1 µg

Lysates/proteins at 0.5 µg per lane.

Secondary
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/5000 dilution

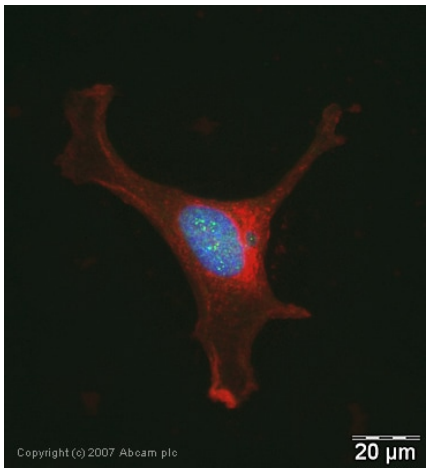
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa
Observed band size: 17 kDa

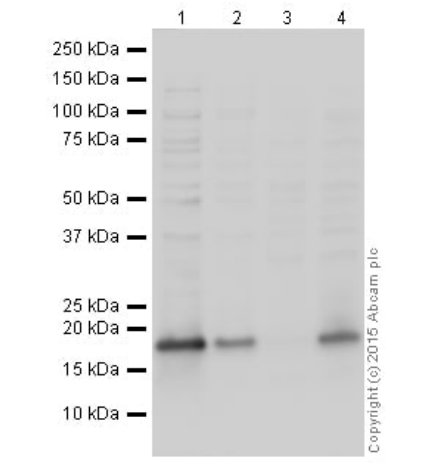
Exposure time: 1 minute

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab6147 overnight at 4°C. Antibody binding was visualised using ECL development solution **ab133406**.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K27, tri methyl K27) antibody [mAbcam 6147] (ab6147)

ICC/IF image of ab6147 stained human HeLa cells. The cells were PFA fixed (10 min), permabilised in TBS-T (20 min) and incubated with the antibody (ab6147, 1µg/ml) for 1h at room temperature. 1%BSA / 10% normal goat serum / 0.3M glycine was used to quench autofluorescence and block non-specific protein-protein interactions. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red). DAPI was used to stain the cell nuclei (blue).



Western blot - Anti-Histone H3 (di methyl K27, tri methyl K27) antibody [mAbcam 6147] (ab6147)

All lanes : Anti-Histone H3 (di methyl K27, tri methyl K27) antibody [mAbcam 6147] (ab6147) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Nuclear Lysate
Lane 2 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 3 : EED-/- mouse ES Whole Cell Lysate
Lane 4 : WT mouse ES Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/5000 dilution

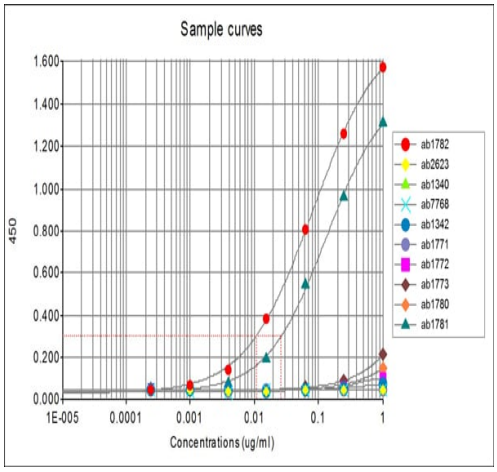
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa
Observed band size: 17 kDa

Exposure time: 10 seconds

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab6147 overnight at 4°C. Antibody binding was visualised using ECL development solution **ab133406**.



ELISA - Anti-Histone H3 (di methyl K27, tri methyl K27) antibody [mAbcam 6147] (ab6147)

All batches of ab6147 are tested in ELISA against peptides to different Histone H3 modifications. Results show strong binding to Histone H3 - tri methyl K27 immunising peptide (**ab1782**) and Histone H3 - di methyl K27, indicating that this antibody specifically recognises both the Histone H3 - tri methyl K27 and di methyl K27 modifications. Binding is detected against the Histone H3 - di methyl K27 modification (>40%) (**ab1781**).

ab2623 - Histone H3 - unmodified

ab1340 - Histone H3 - mono methyl K4

ab7768 - Histone H3 - di methyl K4

ab1342 - Histone H3 - tri methyl K4

ab1771 - Histone H3 - mono methyl K9

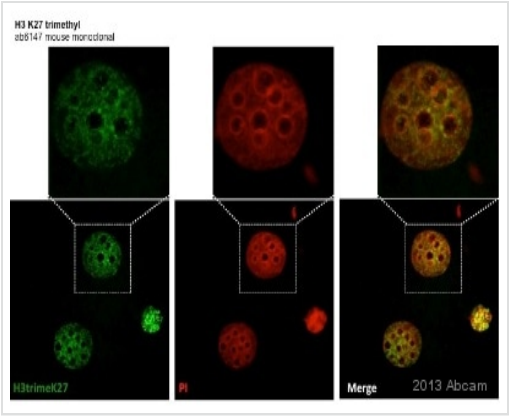
ab1772 - Histone H3 - di methyl K9

ab1773 - Histone H3 - tri methyl K9

ab1780 - Histone H3 - mono methyl K27

ab1781 - Histone H3 - di methyl K27

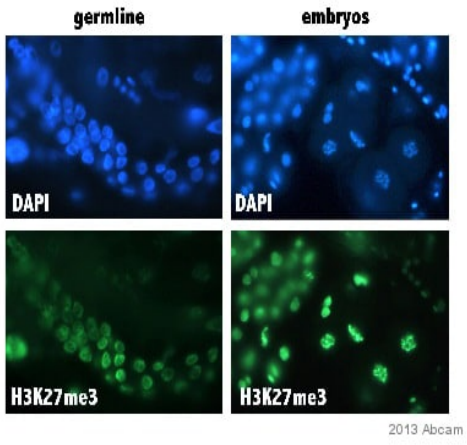
ab1782 - Histone H3 - tri methyl K27



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K27, tri methyl K27) antibody [mAbcam 6147] (ab6147)

Image courtesy of Maki Asami, University of Bath

ICC/IF image of ab6147 stained mouse 2 cell embryo. Cells were fixed with formaldehyde, permeabilized with 0.5% Triton, and incubated with ab6147 at 1/50 for 2 hours at 37°C. Blocking was performed in 5% FCS serum for 30 minutes at 37°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG used at a 1/250 dilution. PI was used to stain the DNA (Red).

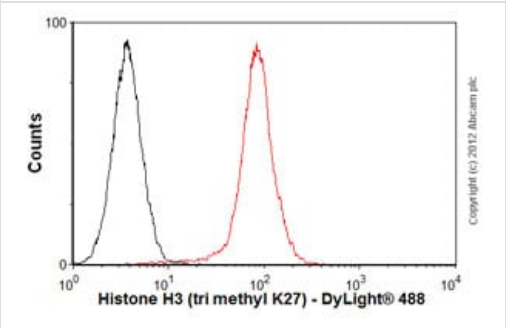


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K27, tri methyl K27) antibody [mAbcam 6147] (ab6147)

This image is courtesy of an Abreview submitted by Carlos Carvalho

Immunofluorescence analysis of *C. elegans* germline cells and gonads, staining Histone H3 (tri methyl K27) with ab6147.

Cells were fixed with methanol, frozen and cracked in liquid nitrogen and blocked with 0.5% BSA for 1 hour at 20°C. Samples were incubated with primary antibody (1/500 in diluent) for 14 hours at 4°C. An FITC-conjugated donkey anti-mouse polyclonal IgG (1/300) was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-Histone H3 (di methyl K27, tri methyl K27) antibody [mAbcam 6147] (ab6147)

Overlay histogram showing HeLa cells stained with ab6147 (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 (ab6147, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was a goat **anti-mouse DyLight® 488** (IgG; H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.cn/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors