abcam

Product datasheet

Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade ab177178

Recombinant RabMAb

8 Abreviews 60 References 15 Images

Overview

Product name	Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade
Description	Rabbit monoclonal [EP16602] to Histone H3 (acetyl K27) - ChIP Grade
Host species	Rabbit
Specificity	ab177178 binds K27ac alone and also when S28 is phosphorylated
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, PepArr, ChIC/CUT&RUN-seq, IHC-P, WB, ChIP, ChIP-sequencing
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: NIH/3T3, C6 and HeLa treated with 500 ng/ml Trichostatin A for 4 hours whole cell lysates. IHC: Human liver cancer tissue, Mouse lung tissue and Rat pancreas tissue. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells. ChIP: Chromatin prepared from HeLa cells. ChIP-seq: Chromatin prepared from HeLa cells. ChIC/CUT&RUN: 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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP16602
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab177178 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
Flow Cyt (Intra)		1/1500.
ICC/IF		1/7000.
PepArr		Use at an assay dependent concentration.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5 µg
IHC-P	★★★★★ (2)	1/1500 - 1/10000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★★★ (2)	1/10000 - 1/100000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
ChIP	★★★★★ (1)	Use 2 µg for 25 µg of chromatin. Use GAPDH ChIP primer pair **ab267832** as positive control.
ChIP-sequencing	★★★★★ (1)	Use 4µg for 10^7 cells.

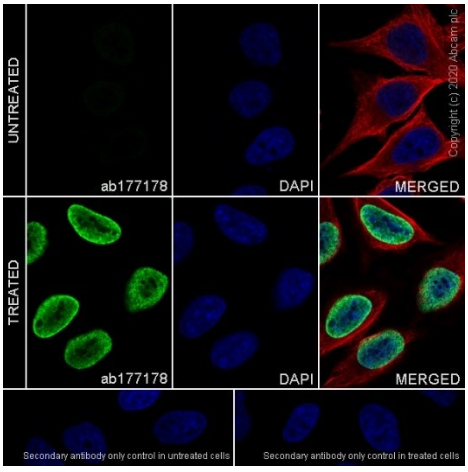
Target

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.

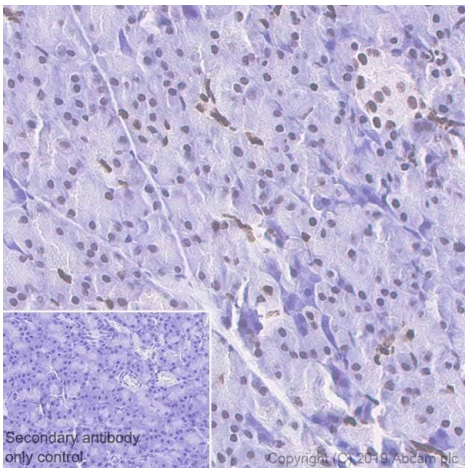
Cellular localization Nucleus. Chromosome.

Images



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178)

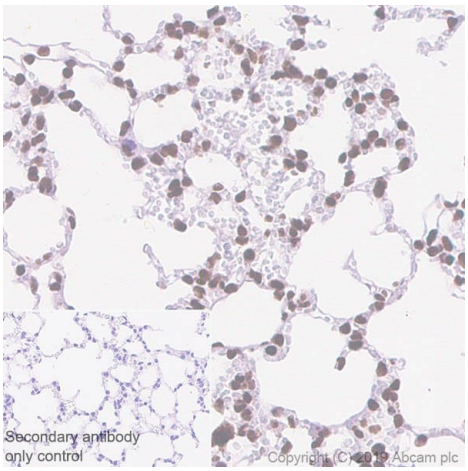
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, labeling Histone H3 (acetyl K27) with ab177178 at 1/8000 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing increased nuclear staining in HeLa cells treated with TSA (500 ng/ml, 4 hours). The nuclear counter stain is DAPI (blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178)

Immunohistochemical analysis of paraffin-embedded rat pancreas tissue labeling Histone H3 (acetyl K27) with ab177178 at 1/10000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) was performed for 20 minutes. Nuclear staining on rat pancreas is observed. Counter stained with Hematoxylin.

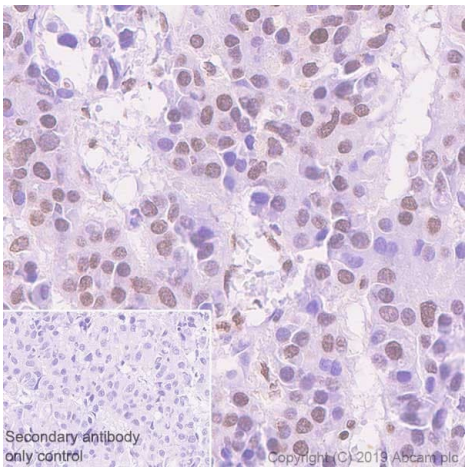
The section was incubated with ab177178 for 30 minutes at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178)

Immunohistochemical analysis of paraffin-embedded mouse lung tissue labeling Histone H3 (acetyl K27) with ab177178 at 1/10000 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) was performed for 20 minutes. Nuclear staining on mouse lung tissue is observed. Counter stained with Hematoxylin.

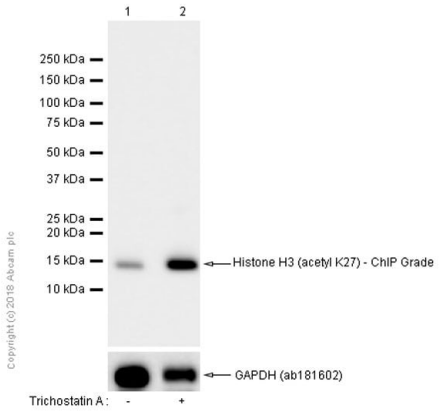
The section was incubated with ab177178 for 30 minutes at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178)

Immunohistochemical analysis of paraffin-embedded human liver cancer tissue labeling Histone H3 (acetyl K27) with ab177178 at 1/1500 dilution, followed by Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) was performed for 20 minutes. Nuclear staining on human liver cancer tissue is observed. Counter stained with Hematoxylin.

The section was incubated with ab177178 for 30 minutes at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND® RX instrument.



Western blot - Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178)

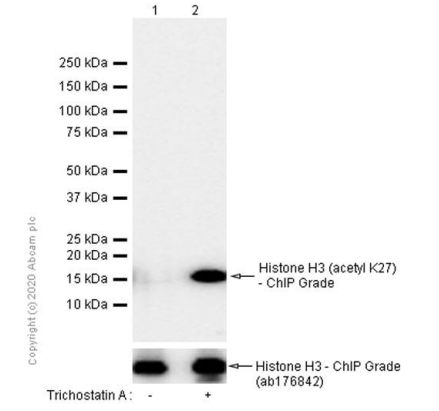
All lanes : Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178) at 1/10000 dilution

Lane 1 : C6 (Rat glial tumor glial cell) whole cell lysate
Lane 2 : C6 (Rat glial tumor glial cell) treated with 500 ng/ml Trichostatin A for 4 hours whole cell lysate

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**)

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



Western blot - Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178)

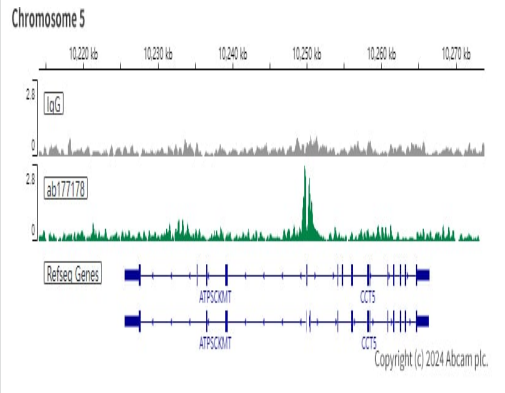
All lanes : Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178) at 1/100000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) treated with 500 ng/ml Trichostatin A for 4 hours whole cell lysate

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

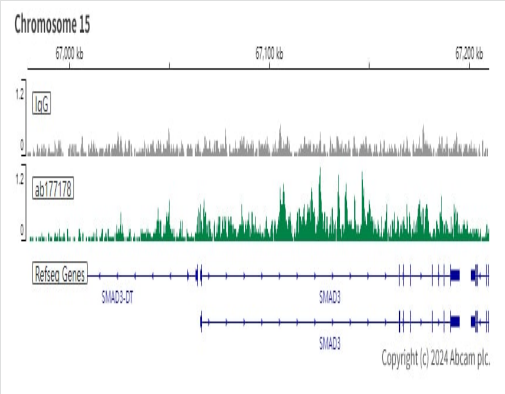
Predicted band size: 15 kDa
Observed band size: 15 kDa



ChIC/CUT&RUN sequencing - Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human epithelial cell line from cervix adenocarcinoma) cells and 5 µg of **ab32129** [Y28]. 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.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



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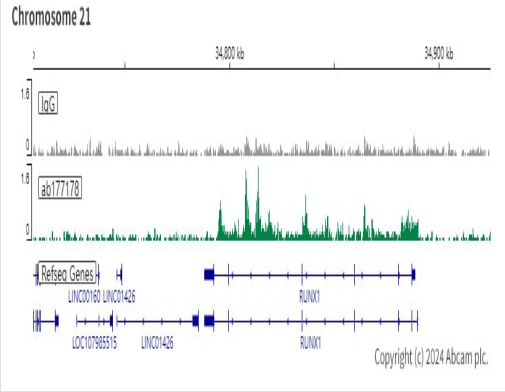

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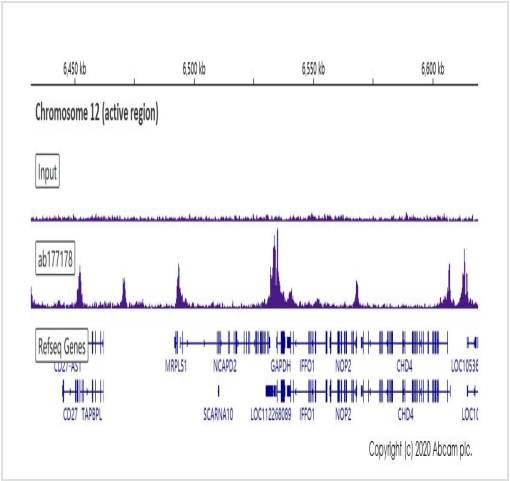
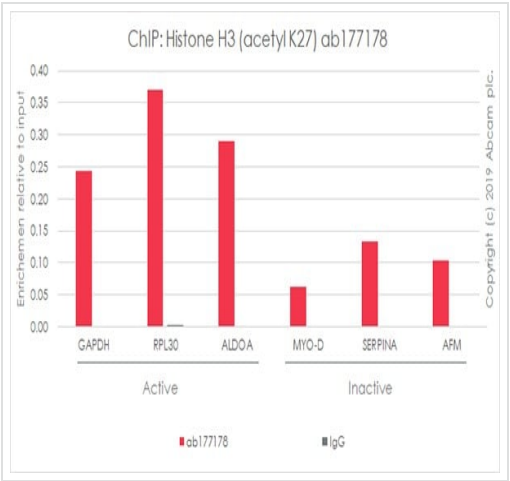
The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



ChIP-sequencing - Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178)

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 of Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178). ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

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

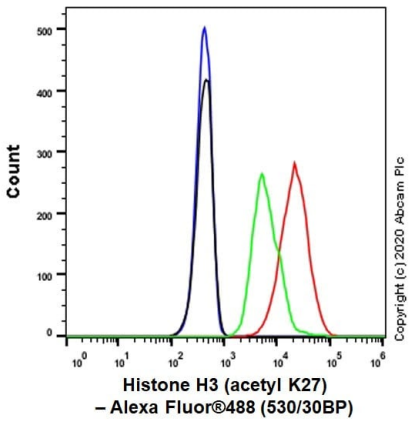


ChIP - Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178)

Chromatin was prepared from HeLa (Human epithelial cell line from cervix adenocarcinoma) 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 ab177178 (blue), and 20µl of Protein A/G Sepharose beads. 2µg of rabbit normal IgG was added to the beads as a control sample (yellow). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci).

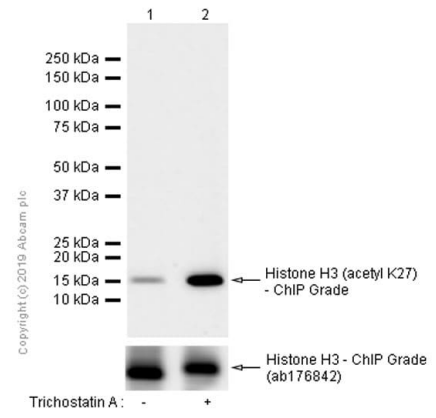
Primers and probes are located in the first kb of the transcribed region.

*http://www.abcam.com/resources?keywords=X%20ChIP%20protocol



Flow Cytometry (Intracellular) - Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) treated (Red)/untreated (Green) with 500ng/ml Trichostatin A for 4 hours with purified ab177178 at 1/1500 dilution. The secondary antibody was Goat anti rabbit IgG (Alexa Fluor®488) at 1/2000 dilution. Rabbit monoclonal IgG (**ab172730**) (Black) was used as the isotype control and cells without incubation with primary antibody and secondary antibody (Blue) were used as unlabeled control.



Western blot - Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178)

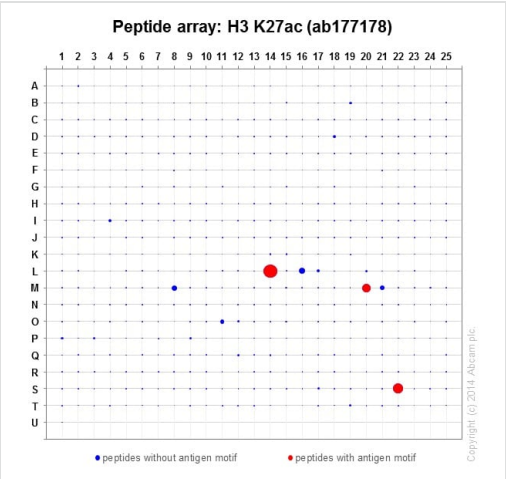
All lanes : Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178) at 1/10000 dilution

Lane 1 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast cell line) treated with 500 ng/ml Trichostatin A for 4 hours whole cell lysate

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**)

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



Peptide Array - Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178)

ab177178 was 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 area under curve when antibody binding values are plotted against the corresponding peptide concentration. Each circle area is normalized to the peptide with the strongest affinity.

The complete dataset, including full list of all peptides and information on the position of each peptide in the diagram, can be downloaded **here**.

Anti-Histone H3 (acetyl K27) antibody [EP16602] - ChIP Grade (ab177178)

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