abcam

Product datasheet

Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade ab192985

リコンビナント RabMAb

13 Abreviews 42 References 画像数 14

製品の概要

製品名	Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade
製品の詳細	Rabbit monoclonal [EPR18607] to Histone H3 (tri methyl K27) - ChIP Grade
由来種	Rabbit
アプリケーション	**適用あり:** IHC-P, ChIP, ICC/IF, WB, PepArr, ELISA, ChIP-sequencing
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: HeLa and NIH/3T3 whole cell lysates; Wild type mouse ES whole cell lysate, IHC-P: Human colon, mouse colon and rat kidney tissues. ICC: HeLa cells. ChIP: Chromatin prepared from HeLa cells, Myo-D ChIP primer pair **ab269261**. ELISA: Histone H3 – unmodified, Histone H3.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	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.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル

クローン名	EPR18607
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab192985の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

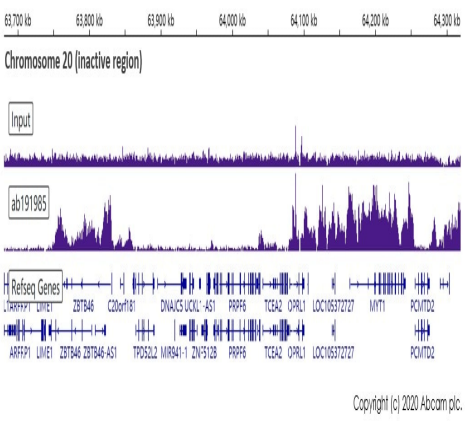
アプリケーション	Abreviews	特記事項
IHC-P	★★★★★ (5)	1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ChIP	★★☆☆☆ (2)	Use 2 µg for 25 µg of chromatin. Use Myo-D ChIP primer pair **ab269261** as positive control.
ICC/IF		1/1000.
WB	★★★☆☆ (4)	1/1000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa). We suggest to use 2% BSA as blocking and antibody dilution buffer. To get stronger band, 1%SDS Hot lysis method is also recommended.
PepArr		Use a concentration of 0.1 µg/ml.
ELISA		Use a concentration of 0.25 µg/ml.
ChIP-sequencing		Use 4µg for 10^7 cells.

ターゲット情報

機能	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.
配列類似性	Belongs to the histone H3 family.
発生段階	Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.
翻訳後修飾	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.
細胞内局在	Nucleus. Chromosome.

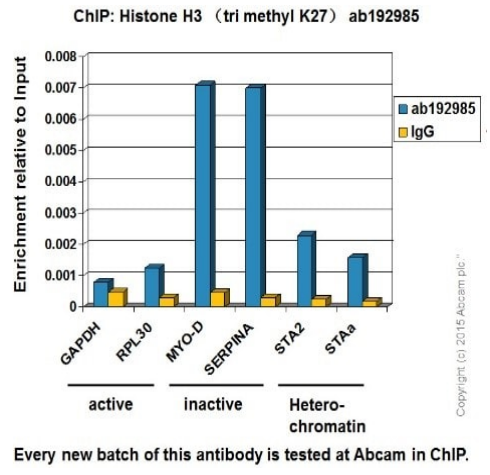
画像



ChIP-sequencing - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985)

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 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985). ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

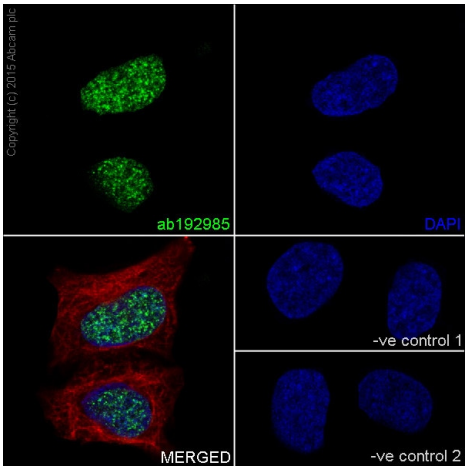
Additional screenshots of mapped reads can be downloaded **here**.



ChIP - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985)

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 25 µg of chromatin, 2 µg of ab192985 (blue), and 20 µl of Anti rabbit IgG sepharose beads. 2 µg of rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

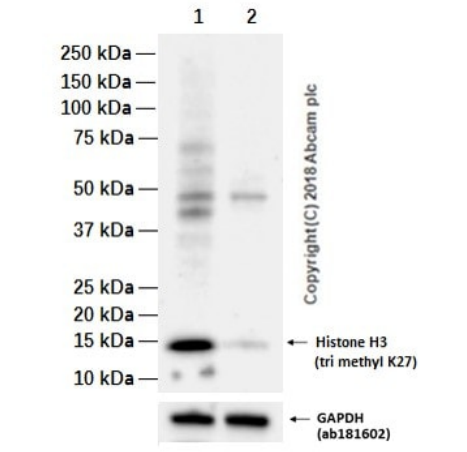


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Histone H3 (tri methyl K27) with ab192985 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:-
-ve control 1: ab192985 at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) secondary antibody at 1/1000 dilution.
-ve control 2: Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Western blot - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985)

All lanes : Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985) at 1/1000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate prepared using 1% SDS hot lysis method
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate prepared using RIPA lysis method

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/5000 dilution

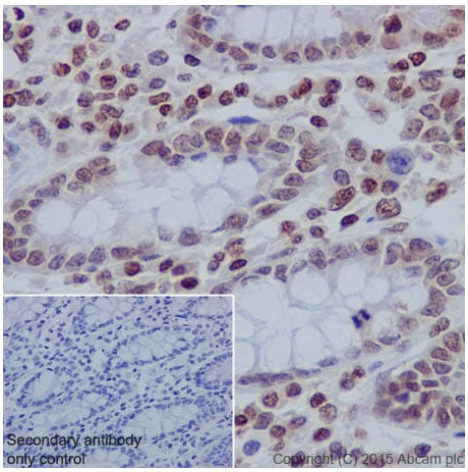
Predicted band size: 15 kDa
Observed band size: 15 kDa

Exposure time: 3 minutes

Blocking/Diluting buffer: 5% NFDM/TBST

For this product, we recommend 1% SDS hot lysis method.

For lysate preparation protocol, please refer to the protocol book in the protocol section or **here (downloadable copy).**

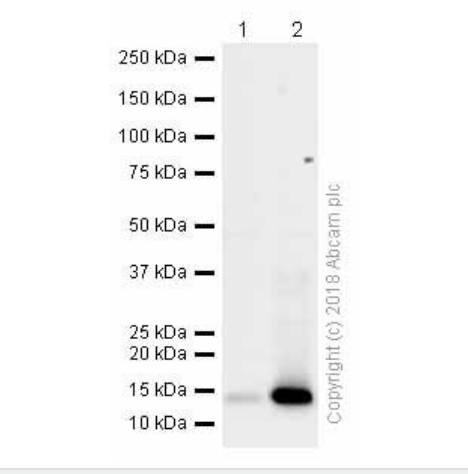


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling Histone H3 (tri methyl K27) with ab192985 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on human colon tissue is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985)

All lanes : Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985) at 1/1000 dilution

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates with 5% NFDM/TBST
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates with 2% BSA/TBST

Lysates/proteins at 15 µg per lane.

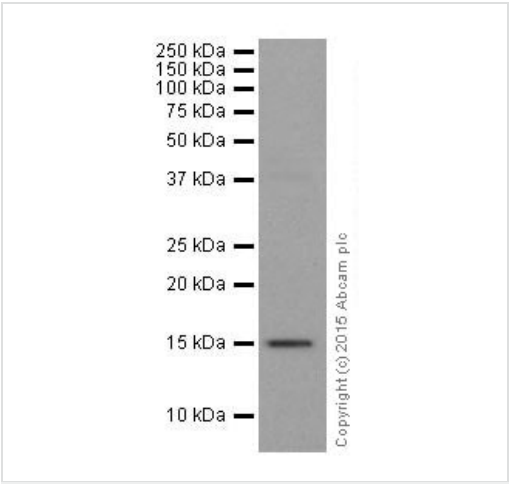
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

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

Exposure time: 40 seconds

We recommend to use 2% BSA as blocking and antibody dilution

buffer.



Western blot - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985)

Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985) at 1/1000 dilution + NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate at 10 µg

Secondary

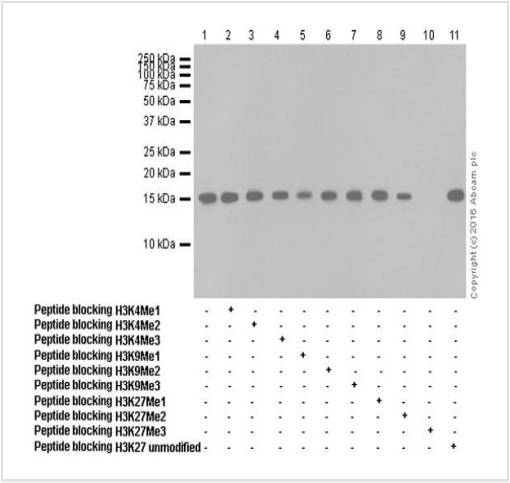
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

Predicted band size: 15 kDa

Observed band size: 15 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% BSA/TBST



Western blot - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985)

All lanes : Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with H3K4Me1 peptide at 5 µg

Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with H3K4Me2 peptide at 5 µg

Lane 4 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with H3K4Me3 peptide at 5 µg

Lane 5 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with H3K9Me1 peptide at 5 µg

Lane 6 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with H3K9Me2 peptide at 5 µg

Lane 7 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with H3K9Me3 peptide at 5 µg

Lane 8 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with H3K27Me1 peptide at 5 µg

Lane 9 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with H3K27Me2 peptide at 5 µg

Lane 10 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with H3K27Me3 peptide at 5 µg

Lane 11 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with H3K27 unmodified peptide at 5 µg

Lysates/proteins at 10 µg per lane.

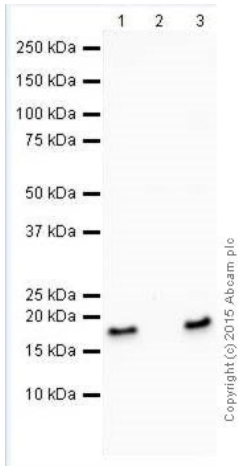
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Developed using the ECL technique.

Predicted band size: 15 kDa

Exposure time: 5 seconds

The antibody is blocked by tri methyl K27 peptide (lane 10) and slightly by di methyl K27 peptide (lane 9, there is 14% cross reactivity with di methyl K27 as determined by ELISA).



Western blot - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985)

All lanes : Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma)
Lane 2 : EED-/- mouse whole cell lysate
Lane 3 : Wild type mouse ES whole cell lysate

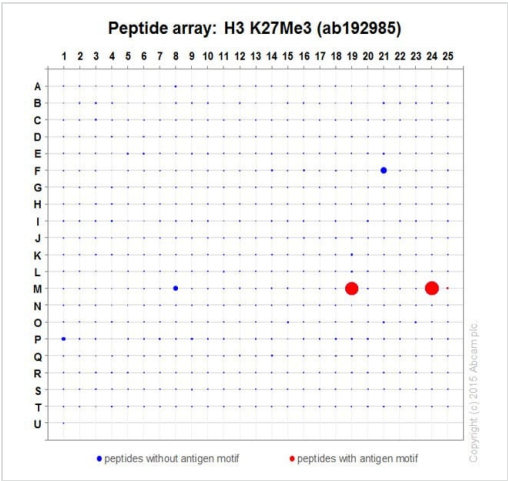
Lysates/proteins at 10 µg per lane.

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

Developed using the ECL technique.

Predicted band size: 15 kDa

Exposure time: 8 minutes



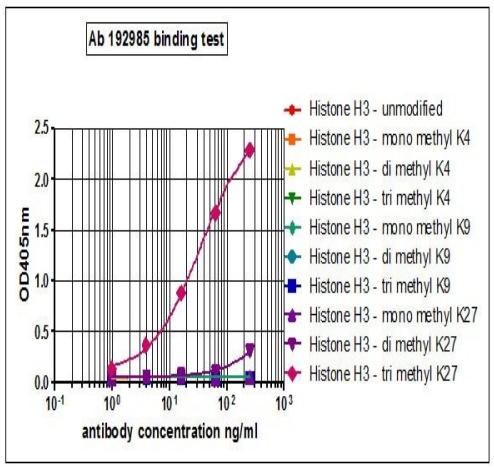
Peptide Array - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985)

Peptide array analysis was performed using ab192985 at a concentration of 0.1 µg/ml, followed by Goat Anti-Rabbit IgG, (H+L), Fluo 647nm conjugated secondary antibody at a 1/50,000 dilution.

ab192985 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.

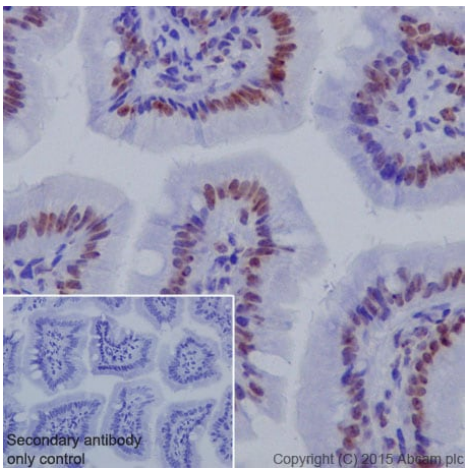
The complete dataset, including full list of all peptides and information on the position of each peptide in the diagram, can be downloaded **here**.



ELISA - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985)

ELISA analysis was performed on 1 µg/ml of antigen using ab192985 at a concentration range of 0-0.25 µg/ml, followed by Alkaline Phosphatase-conjugates AffiniPure Goat anti-rabbit IgG (H&L) secondary antibody at a 1/2,500 dilution.

All batches of ab192985 are tested in ELISA against peptides to different Histone H3 modifications. Results show strong binding to Histone H3 - tri methyl K27 immunizing peptide, indicating that this antibody specifically recognizes the Histone H3 - tri methyl K27 modification. Weak binding **(14%)** was also detected against H3 - di methyl K27 modification.

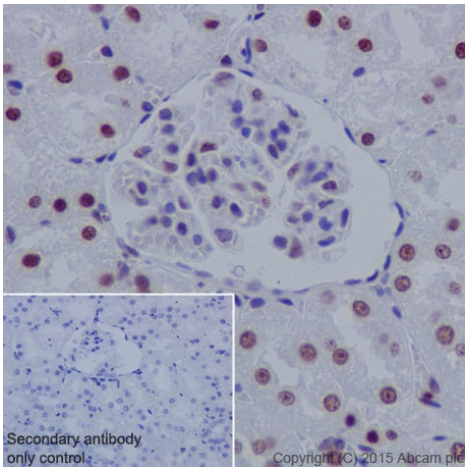


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling Histone H3 (tri methyl K27) with ab192985 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on mouse colon tissue is observed. Counterstained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

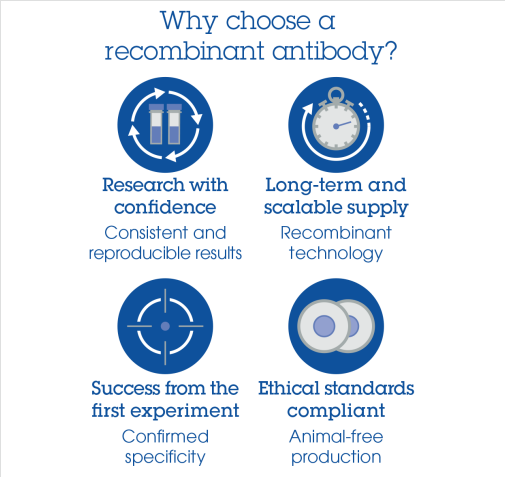


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (ab192985)

Immunohistochemical analysis of paraffin-embedded rat kidney tissue labeling Histone H3 (tri methyl K27) with ab192985 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution. Nuclear staining on rat kidney tissue is observed. Counterstained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Anti-Histone H3 (tri methyl K27) antibody

[EPR18607] - ChIP Grade (ab192985)

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