abcam

Product datasheet

Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade ab176840

リコンビナント RabMAb

★★★★★ 2 Abreviews 27 References 画像数 10

製品の概要

製品名	Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade
製品の詳細	Rabbit monoclonal [EPR17899] to Histone H3.3 - ChIP Grade
由来種	Rabbit
特異性	The immunogen sequence used for this antibody is 100% identical to the protein sequence shown for H3.X and H3.Y in Szenker et al., 2011 (PubMed ID 21263457). These H3.X and H3.Y protein sequences have not been reviewed by UniProt. The immunogen sequense is also 100% homologous to Histone H3.6. For further information on this antibody, please contact our technical support team.
アプリケーション	**適用あり:** ChIP-sequencing, ICC/IF, ChIP, IHC-P, WB, Dot blot
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. IHC-P: Human colon, Mouse stomach and Rat colon tissues. ICC/IF: HeLa cells. ChIP: Chromatin prepared from HeLa cells. ChIP-seq: Chromatin prepared from HeLa cells.
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	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 0.05% BSA, 40% Glycerol

精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR17899
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ab176840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
ChIP-sequencing		Use 4 µg for 30 µg of chromatin.
ICC/IF	★★★★★ (1)	1/1000.
ChIP		Use 2 µg for 25 µg of chromatin.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★★★ (1)	1/1000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
Dot blot		1/1000.

ターゲット情報

機能 Variant histone H3 which replaces conventional H3 in a wide range of nucleosomes in active genes. Constitutes the predominant form of histone H3 in non-dividing cells and is incorporated into chromatin independently of DNA synthesis. Deposited at sites of nucleosomal displacement throughout transcribed genes, suggesting that it represents an epigenetic imprint of transcriptionally active chromatin. 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.

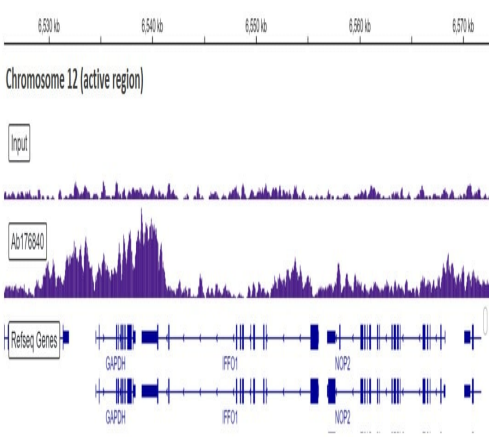
発生段階 Expressed throughout the cell cycle independently of DNA synthesis.

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.
Specifically enriched in modifications associated with active chromatin such as methylation at Lys-5 (H3K4me), Lys-37 and Lys-80. 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), which are linked to gene repression, are underrepresented. 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. Phosphorylation on Ser-32 (H3S31ph) is specific to regions bordering centromeres in metaphase chromosomes.
Ubiquitinated. Monoubiquitinated by RAG1 in lymphoid cells, monoubiquitination is required for V(D)J recombination.

細胞内局在 Nucleus. Chromosome.

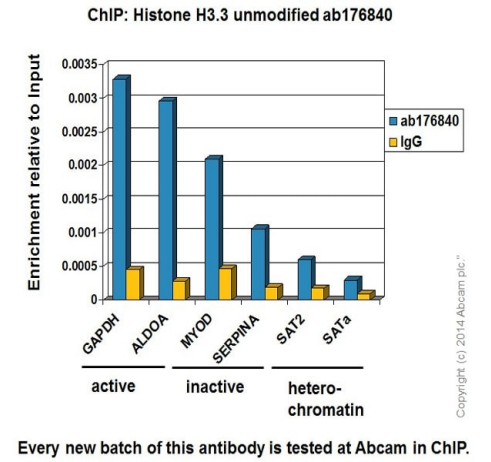
画像



ChIP-sequencing - Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade (ab176840)

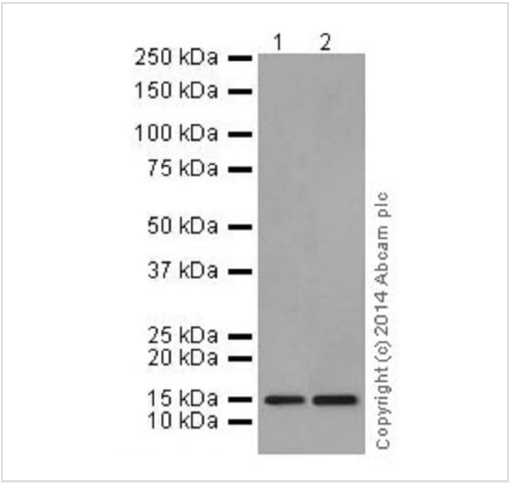
Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 30 µg of chromatin and 4 µg of Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade (ab176840). ChIP DNA was sequenced on the Illumina NextSeq 500 to a depth of 30 million reads. ChIP-Seq validation performed by Active Motif, Carlsbad, CA.

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



ChIP - Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade (ab176840)

Chromatin was prepared from HeLa (Human epithelial cells 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 ab176840 (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).



Western blot - Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade (ab176840)

All lanes : Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade (ab176840) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate
Lane 2 : NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate

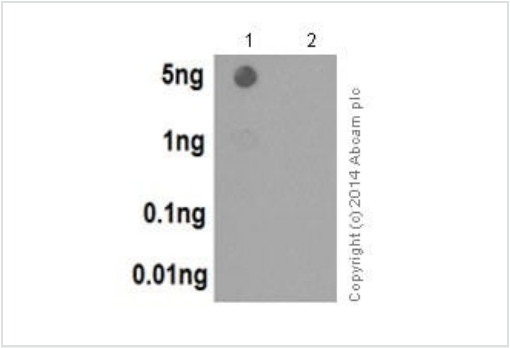
Lysates/proteins at 10 µg per lane.

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

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

Exposure time: 3 minutes

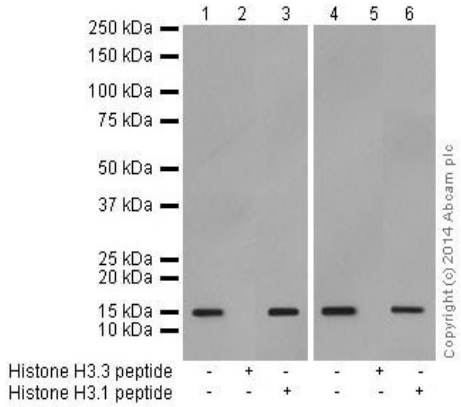
Blocking/Dilution buffer: 5% NFDM/TBST.



Dot Blot - Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade (ab176840)

Dot blot analysis of Histone H3.3 peptide (Lane 1), and Histone H3.1 peptide (Lane 2), labeled using ab176840 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated secondary antibody at 1/1000 dilution.

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade (ab176840)

All lanes : Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade (ab176840) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate
Lane 2 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate with Histone H3.3 peptide
Lane 3 : HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate with Histone H3.1 peptide
Lane 4 : NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate
Lane 5 : NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate with Histone H3.3 peptide
Lane 6 : NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate with Histone H3.1 peptide

Lysates/proteins at 10 µg per lane.

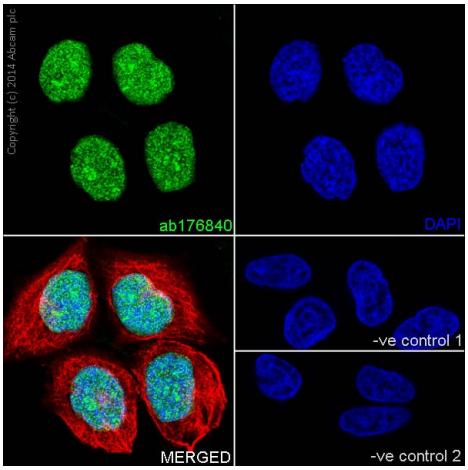
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

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

Exposure time: 3 minutes

Blocking experiment by pre-incubating Histone H3.3 peptide with the antibody in lanes 2 and 5 and Histone H3.1 peptide in lanes 3 and 6 to show the antibody specificity against Histone H3.3.

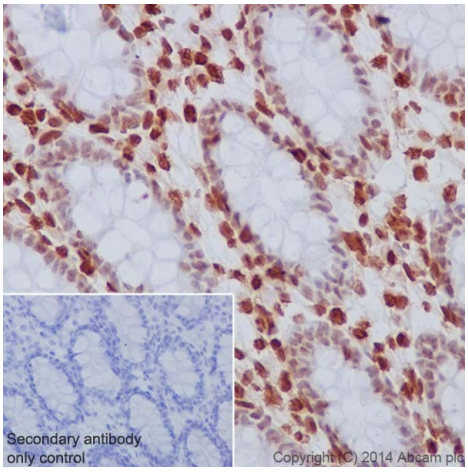
Blocking/Dilution buffer: 5% NFDM/TBST.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade (ab176840)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Histone H3.3 with ab176840 at 1/1000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:
-ve control 1: ab176840 at 1/1000 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.

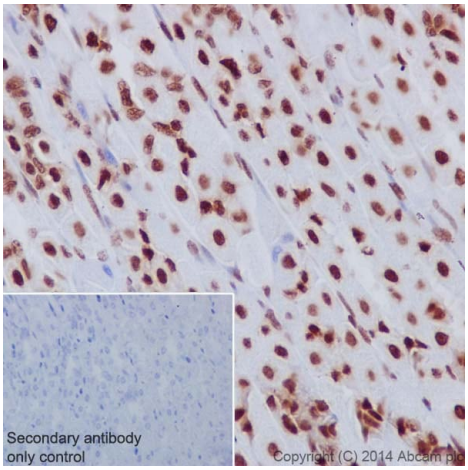


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade (ab176840)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Histone H3.3 with ab176840 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution. Nucleus 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.

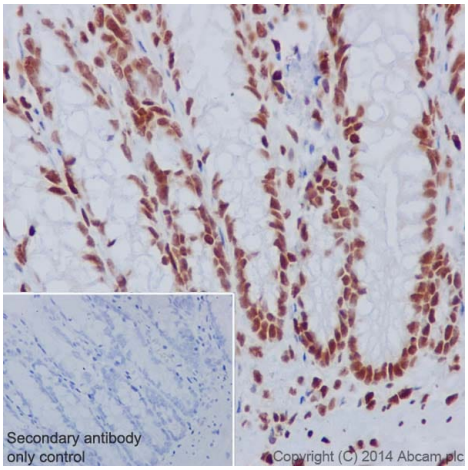


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade (ab176840)

Immunohistochemical analysis of paraffin-embedded Mouse stomach tissue labeling Histone H3.3 with ab176840 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution. Nucleus staining on mouse stomach 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.

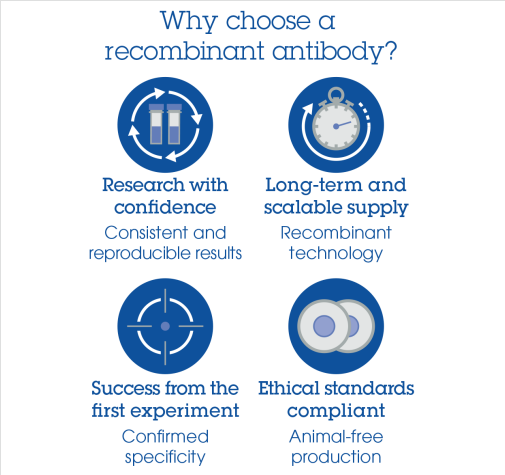


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade (ab176840)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling Histone H3.3 with ab176840 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) secondary antibody at 1/500 dilution. Nucleus staining on Rat 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.



Anti-Histone H3.3 antibody [EPR17899] - ChIP Grade (ab176840)

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