abcam

Product datasheet

Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade ab5103

14 Abreviews 82 References 画像数 7

製品の概要

製品名	Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade
製品の詳細	Rabbit polyclonal to Histone H3 (citrulline R2 + R8 + R17) - ChIP Grade
特異性	ab5103 detects a 17 kDa band in single lane Western Blot. Peptide inhibition in Western Blot hasn't been processed. Modification specificity is determined by Peptide Array. ab5103 binds strongly to Histone H3 citrulline 2 + 8 + 17 peptide.
アプリケーション	**適用あり:** ICC/IF, PepArr, IHC-Fr, Flow Cyt, ChIP/Chip, WB, ChIP
種交差性	**交差種:** Mouse, Rat, Rabbit, Cow, Human, Monkey **交差が予測される動物種:** a wide range of other species
免疫原	Synthetic peptide corresponding to Human Histone H3 aa 1-100 (citrulline R2 + R8 + R17) conjugated to Keyhole Limpet Haemocyanin (KLH). Also SwissProt: P84243, Q71DI3, Q16695, Q6NXT2. Database link: P68431 (Peptide available as ab32876)
ポジティブ・コントロール	This antibody gave a positive signal in HL60 Whole Cell Lysate - DMSO and Calcium Ionophore treated. In WB ab5103 only recognizes human or bovine histone H3 when PADI4 and calcium are added.

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 or -80°C. Avoid freeze / thaw cycle.
バッファー	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.
精製度	Immunogen affinity purified

ポリ/モノ	ポリクローナル
アイソタイプ	IgG

アプリケーション

Our Abpromise guarantee covers the use of **ab5103** in the following tested applications.

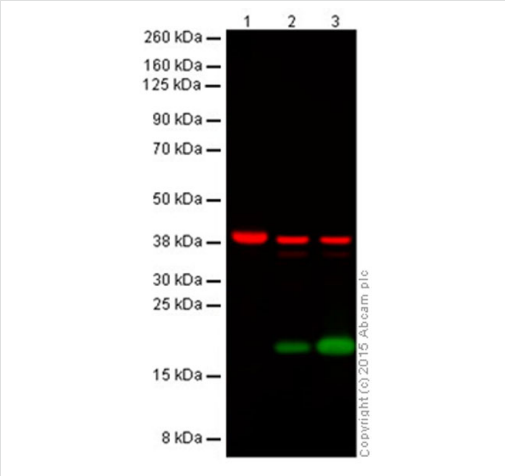
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

アプリケーション	Abreviews	特記事項
ICC/IF	★★★★★	Use at an assay dependent concentration. PubMed: 20733033
PepArr		Use a concentration of 0.2 - 0.02 µg/ml.
IHC-Fr	★★★★★	Use at an assay dependent concentration.
Flow Cyt		Use at an assay dependent concentration. ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.
ChIP/Chip		Use at an assay dependent concentration.
WB	★★★☆☆	Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa). Abcam recommends using 3-5% milk as the blocking agent We recommend Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody
ChIP		Use at an assay dependent concentration.

ターゲット情報

関連性	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- modifications of histones, also called histone code, and nucleosome remodeling. P68431 and Q71DI3 are expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation. Q16695 is expressed in tesicular cells. Q6NXT2 is specifically expressed in the seminiferous tubules of testis and this Hominid-specific H3.5/H3F3C preferentially colocalizes with euchromatin, and it is associated with actively transcribed genes. P84243 is a 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. Post-translational modification. Citrullination at Arg-9 (H3R8ci) and/or Arg-18 (H3R17ci) by PADI4 impairs methylation and represses transcription.
細胞内局在	Nuclear

画像



Western blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade (ab5103)

All lanes : Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade (ab5103) at 0.2 µg/ml

Lane 1 : HL60 whole cell lysate (negative control)
Lane 2 : HL60 whole cell lysate + DMSO (solvent control)
Lane 3 : HL60 whole cell lysate + DMSO + Calcium Ionophore (positive control)

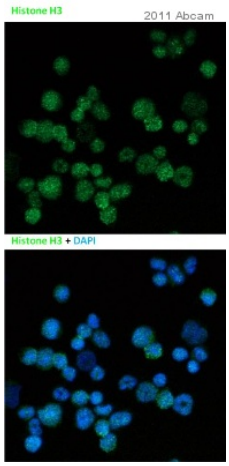
Lysates/proteins at 20 µg per lane.

Secondary
Goat anti Rabbit IR680 at 1/10000 dilution

Performed under reducing conditions.

Predicted band size : 15 kDa
Observed band size : 17 kDa
Loading Control: GAPDH

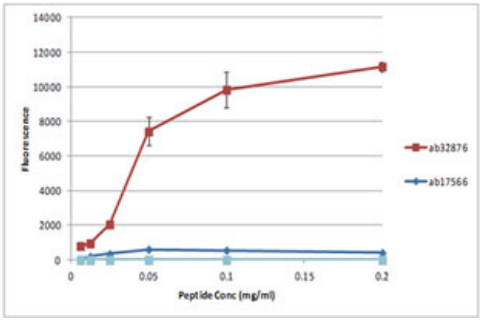
This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab5103 overnight at 4°C. Antibody binding was detected using Goat anti Rabbit IR680 secondary at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade (ab5103)

This image is courtesy of an Anonymous Abreview.

ab5103 staining Histone H3 (citrulline 2 + 8 + 17) in Mouse bone marrow cells by Immunocytochemistry/ Immunofluorescence. Cells were fixed in formaldehyde and permeabilized in 0.1% Triton X-100 prior to blocking in 5% Goat serum for 2 hours at 25°C. The primary antibody was diluted 1/250 in PBS and incubated with the sample for 12 hours at 4°C. The secondary antibody was Alexa Fluor® 488-conjugated Goat anti-Rabbit polyclonal, diluted 1/500.
Nuclei were counterstained blue with DAPI.

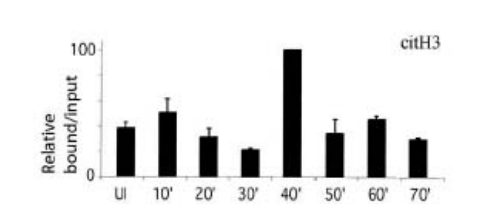


Peptide Array - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (ab5103)

All batches of ab5103 are tested in Peptide Array against peptides to different Histone H3 modifications. Six dilutions of each peptide are printed on to the Peptide Array in triplicate and results are averaged before being plotted on to a graph. Results show strong binding to Histone H3 - citrulline 2 + 8 + 17 peptide (ab32876), indicating that this antibody specifically recognises the Histone H3 - citrulline 2 + 8 + 17 modifications.

ab32876 - Histone H3 - citrulline 2 + 8 + 17

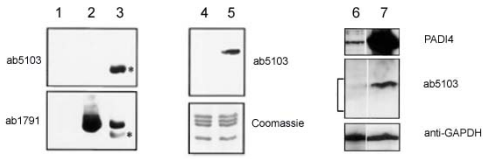
ab17566 - Histone H3 - unmodified



ChIP - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade (ab5103)

Cuthbert, G.L. et al, (2004) Cell 118, 545-553

Chromatin immunoprecipitation using ab5103 on the pS2 promoter. Times are after stimulation by estrogen (UI).



Western blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade (ab5103)

Cuthbert, G.L. et al, (2004) Cell 118, 545-553

Predicted band size : 15 kDa

Cuthbert, G.L. et al, (2004) Cell 118, 545-553

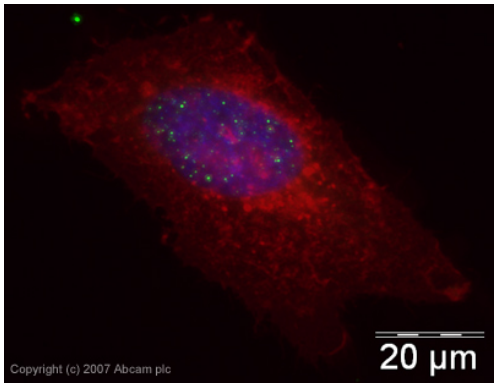
Rabbit polyclonal to Histone H3 (citrulline 2 + 8 + 17) used at 1/2000 dilution, after blocking with TBST 5% BSA. Purified histones run out with approximately 250 ng of each histone.

Lanes 1-3 contain Histone H3 (250 ng per lane)
Lane 1: PADI4 + Calcium
Lane 2: H3 + PADI4
Lane 3: H3 + PADI4 + Calcium

Lanes 4-5 contain bulk histones (250 ng per lane)
Lane 4: PADI4
Lane 5: PADI4 + Calcium

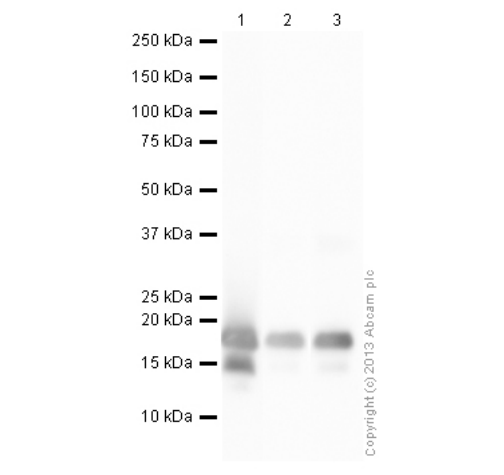
Lane 6: MCF7 cell extract
Lane 7: MCF7 cell extract (HA-PADI4)

Secondary antibody : anti-rabbit HRP from Sigma.
In WB ab5103 only recognizes human or bovine histone H3 when PADI4 and calcium are added.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade (ab5103)

ICC/IF image of ab5103 stained human HeLa cells. The cells were PFA fixed (10 min), permabilised in TBS-T (20 min) and incubated with the antibody (ab5103, 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-rabbit 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 (citrulline R2 + R8 + R17) antibody - ChIP Grade (ab5103)

All lanes : Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade (ab5103) at 1 µg/ml

Lane 1 : HL60 (Human Caucasian promyelocytic leukaemia) DMSO and Calcium Ionophore treated Whole Cell Lysate with with 5% BSA
Lane 2 : HL60 (Human Caucasian promyelocytic leukaemia) DMSO and Calcium Ionophore treated Whole Cell Lysate with with 5% milk
Lane 3 : HL60 (Human Caucasian promyelocytic leukaemia) DMSO and Calcium Ionophore treated Whole Cell Lysate with with 3% milk

Lysates/proteins at 10 µg per lane.

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution
Developed using the ECL technique

Performed under reducing conditions.

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

Exposure time : 30 seconds

Abcam recommends using milk as the blocking agent. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above .

Blots were developled with Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody

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