abcam

Product datasheet

Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade ab32388

Recombinant RabMAb

3 Abreviews 13 References 10 Images

Overview

Product name	Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade
Description	Rabbit monoclonal [E191] to Histone H3 (phospho S28) - ChIP Grade
Host species	Rabbit
Specificity	This antibody detects Histone H3 and Histone H3.3 when phosphorylated on Serine 28. It does not detect H3.3 when phosphorylated on Serine 31.
Tested applications	**Suitable for:** WB, IHC-P, Flow Cyt (Intra), ChIP, Dot blot, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat, Guinea pig
Immunogen	Synthetic peptide within Human Histone H3 aa 1-100 (phospho S28). The exact sequence is proprietary. Database link: **Q16695**
Positive control	NIH 3T3 cell lysate, lymphoma tissue. IHC-P: Human normal colon FFPE tissue sections. ChIP: Chromatin prepared from HeLa cells. IP: HeLa
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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20

	Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E191
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32388 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
WB	★★★☆☆ (2)	1/2000. Predicted molecular weight: 17 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/100 - 1/1200. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ChIP		Use 5 µg for 25 µg of chromatin.
Dot blot		Use at an assay dependent concentration.
ICC/IF		1/250.
IP		1/60.

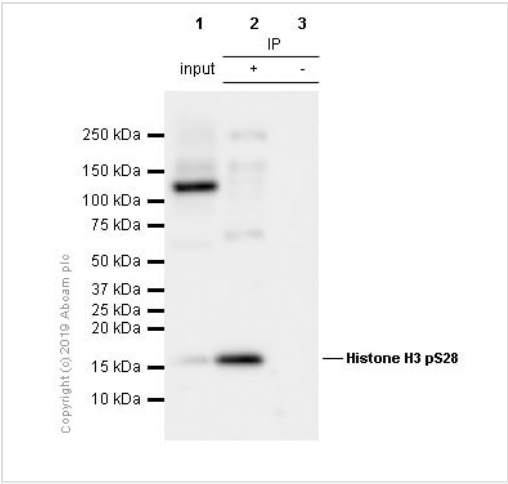
Target

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



Immunoprecipitation - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Immunoprecipitation dilution was 1/60.

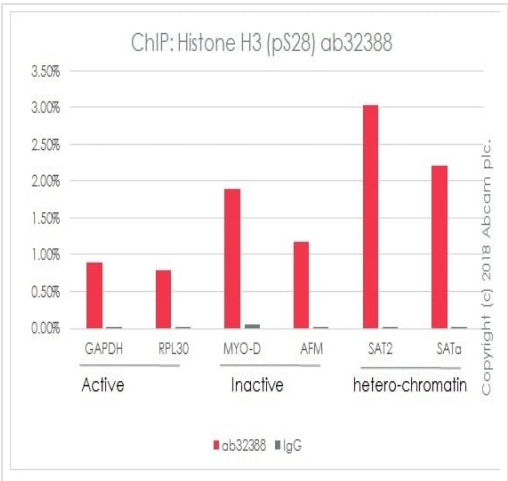
Western blot was performed on the immunoprecipitate using ab32388 at 1/500 dilution (2.444 µg/ml). VeriBlot for IP secondary antibody (HRP) (**ab131366**) at 1:1000 dilution.

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

All lanes :

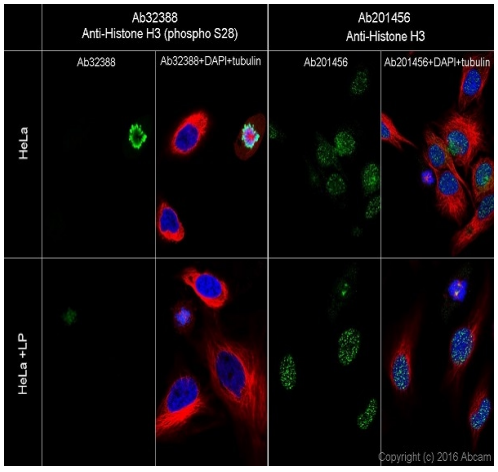
Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) treated with 0.5µM Nocodazole for 24h whole cell lysate at 10 µg
Lane 2 : ab32388 IP in Nocodazole treated HeLa whole cell lysate
Lane 3 : Rabbit monoclonal IgG (**ab172730**) instead of ab32388 in nocodazole treated HeLa whole cell lysate

Observed band size: 17 kDa



ChIP - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25 µg of chromatin, 5 µg of ab32388 (red), and 20 µl of Protein A/G sepharose beads. Rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach). Primers and probes are located in the first kb of the transcribed region.

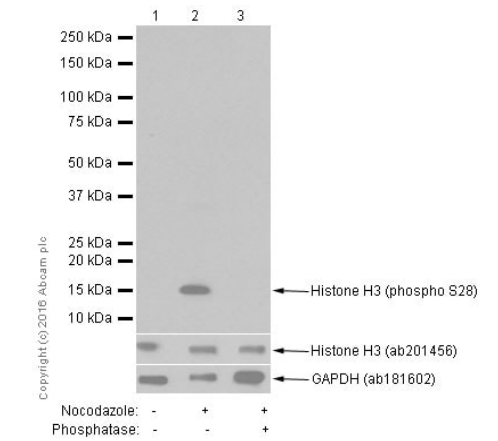


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% tritonX-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, LP starved and non-starved, labeling anti-Histone H3 (phospho S28) with Ab32388 at 1/100 dilution followed by Goat anti-Rabbit secondary IgG AlexaFluor®488 (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear staining on M phase of HeLa cells, then the signal decreased after LP treatment.

For the pan antibody, there was no great difference after LP treatment. The data showed mostly nuclear staining



Western blot - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

All lanes : Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : Whole cell lysate from HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, treated with 100ng/ml nocodazole for 18 hours
Lane 3 : Whole cell lysate from HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, treated with 100ng/ml nocodazole for 18 hours. Membrane incubated with phosphatase

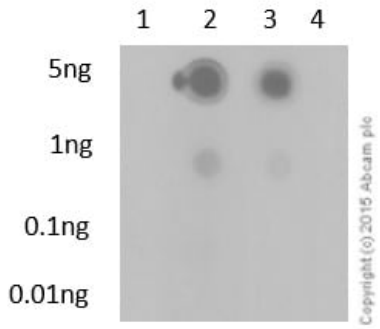
Lysates/proteins at 15 µg per lane.

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

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

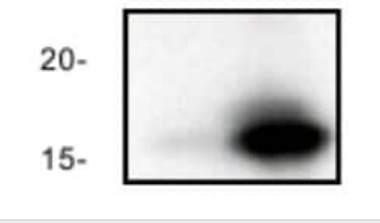
Exposure time: 15 seconds

Blocking/dilution buffer: 2% BSA/TBST



Dot Blot - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Dot blot performed using ab32388 at a dilution of 1/100. Lane 1 - Unmodified H3 peptide. Lane 2 - H3S28ph peptide. Lane 3 - H3.3S28ph peptide. Lane 4 - H3.3S31ph peptide. A HRP conjugated goat anti-rabbit (H+L) was used as the secondary antibody at a dilution of 1/2500. The exposure time was 3 minutes and the dilution and blocking buffer used were 5% NFDM/TBST.

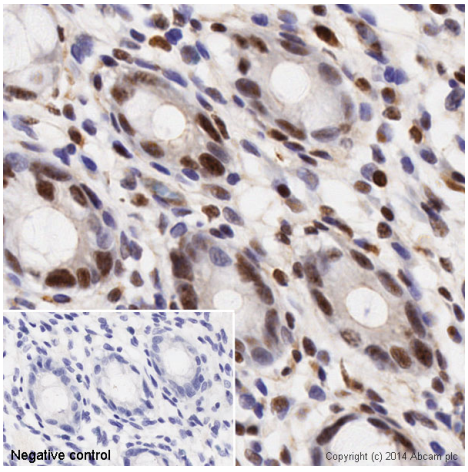


Western blot - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

All lanes : Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388) at 1/2000 dilution

Lane 1 : NIH 3T3 cell lysate -untreated
Lane 2 : NIH 3T3 cell lysate -treated with FBS + CalA.

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

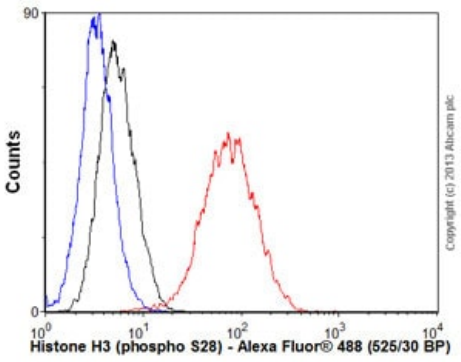


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

IHC image of ab32388 staining Histone H3 in Human normal colon formalin fixed paraffin embedded tissue* sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab32388, 0.1ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the negative control (shown on the inset).

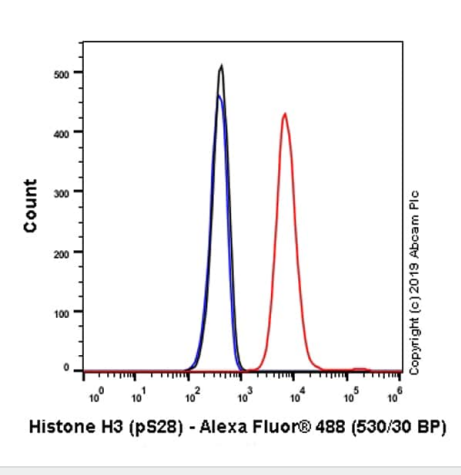
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Flow Cytometry (Intracellular) - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Overlay histogram showing HeLa cells stained with ab32388 (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 (ab32388, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Flow Cytometry (Intracellular) - Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

Overlay histogram showing HeLa cells stained with ab32388 (red line). The cells were fixed with 4% paraformaldehyde and then permeabilized with 90% methanol. 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 (ab32388, 1/1200 dilution, 1.02 µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (**ab172730**) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

Anti-Histone H3 (phospho S28) antibody [E191] - ChIP Grade (ab32388)

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