Anti-Histone H3 antibody ab70550

5 Images

Overview

Product name	Anti-Histone H3 antibody
Description	Rabbit polyclonal to Histone H3
Tested applications	IHC-P, ICC/IF, WB, IP
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat, Rabbit, Chicken, Cow, Pig, Xenopus laevis, Fruit fly (Drosophila melanogaster), Zebrafish, Orangutan, Xenopus tropicalis
Immunogen	Synthetic peptide corresponding to a region between residue 100 and the C-terminus (residue 135) of human Histone H3. (NP_003520.1)
Positive control	Histones isolated from 293T cells. IHC-P: Human normal colon FFPE tissue sections. ICC/IF: HeLa cells

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	Preservative: 0.09% Sodium azide Constituents: 0.1% BSA, Tris buffered saline
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab70550** in the following tested applications.

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

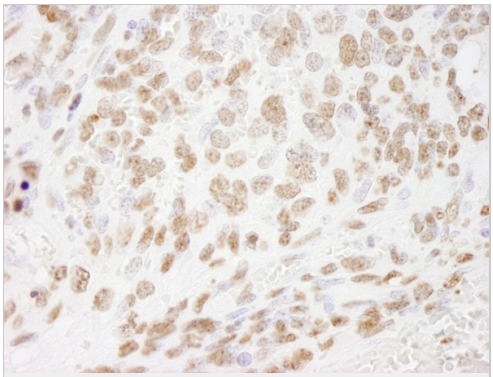
Application	Abreviews	Notes
IHC-P		Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF		Use a concentration of 2.5 µg/ml.
WB		1/2000 - 1/10000. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).
IP		Use at 2-5 µg/mg of lysate.

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

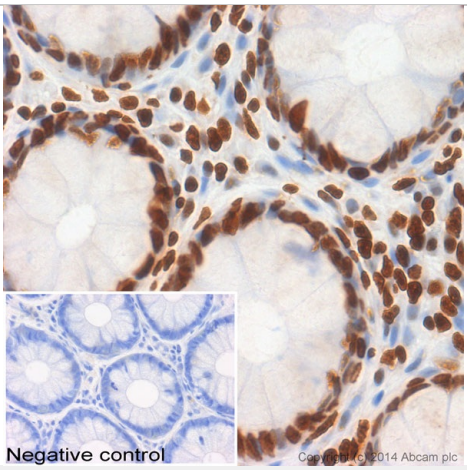
	(H3R2me2a) by PRMT6 is linked to gene repression and is mutually exclusive with H3 Lys-5 methylation (H3K4me2 and H3K4me3). H3R2me2a is present at the 3' of genes regardless of their transcription state and is enriched on inactive promoters, while it is absent on active promoters. Methylation at Lys-5 (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.

Anti-Histone H3 antibody images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 antibody (ab70550)

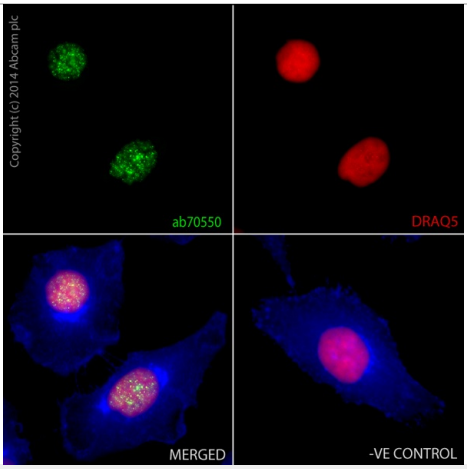
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse teratoma tissue labelling Histone H3 with ab70550 at 1/1000 (0.2µg/ml). Detection: DAB.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 antibody (ab70550)

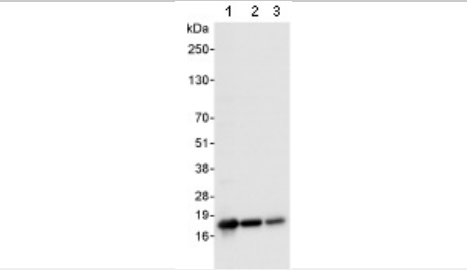
IHC image of ab70550 staining Histone H3 in human colon formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab70550, 1µg/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.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 antibody (ab70550)

ab70550 staining Histone H3 in HeLa cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab70550 at 2.5µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat anti-rabbit AlexaFluor®488 secondary (ab150077) at 2 µg/ml (shown in green). AlexaFluor®350 WGA was used at a 1/200 dilution and incubated for 1h with the cells, to label plasma membranes (shown in blue). Nuclear DNA was labelled in red with 1.25 µM DRAQ5™ (ab108410), which was added to the secondary antibody mixture. A secondary only negative control is displayed, which indicates that the Histone H3 staining observed is due to primary antibody specificity and not to unspecific binding of the secondary antibody to the cells.



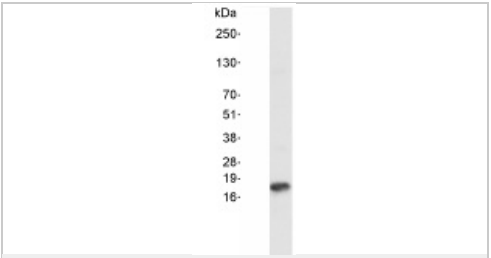
Western blot - Histone H3 antibody (ab70550)

All lanes : Anti-Histone H3 antibody (ab70550) at 0.04 µg/ml

Lane 1 : Histones isolated from 293T cells at 50 µg
Lane 2 : Histones isolated from 293T cells at 15 µg
Lane 3 : Histones isolated from 293T cells at 5 µg

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

Exposure time : 1 second



Immunoprecipitation - Histone H3 antibody (ab70550)

Detection of Human Histone H3 by Western Blot of Immunprecipitate. ab70550 at 1µg/ml staining Histone H3 isolated from 293T cells, immunoprecipitated using ab70550 at 3µg/mg lysate (1 mg/IP; 20% of IP loaded/lane). Detection: Chemiluminescence with exposure time of 1 second.

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