abcam

Product datasheet

Anti-Histone H3 (phospho S10 + T11) antibody [E173] ab32107

Recombinant RabMAb

4 Abreviews 49 References 10 Images

Overview

Product name	Anti-Histone H3 (phospho S10 + T11) antibody [E173]
Description	Rabbit monoclonal [E173] to Histone H3 (phospho S10 + T11)
Host species	Rabbit
Specificity	This antibody detects Histone H3 phosphorylated on both Serine 10 and Threonine 11. However, the antibody shows higher affinity for phosphorylated Serine 10 than for phosphorylated Threonine 11. This was validated by ELISA, Dot Blot and WB peptide blocking experiments.
Tested applications	**Suitable for:** ELISA, Dot blot, ICC/IF, WB, IHC-P, IP **Unsuitable for:** Flow Cyt
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat, Drosophila melanogaster, a wide range of other species
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa whole cell lysate (**ab150035**) treated with Calyculin A. IHC-P: Lymphoma tissue. ICC/IF: HeLa cells IP: HeLa treated with calyculin A whole cell lysate.
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: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E173
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32107 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
ELISA		Use at an assay dependent concentration.
Dot blot		1/1000.
ICC/IF	★★★★★ (1)	1/250 - 1/1000.
WB	★★★★★ (1)	1/2500. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).
IHC-P	★★★★★ (2)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		1/50.

Application notes Is unsuitable for Flow Cyt.

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.

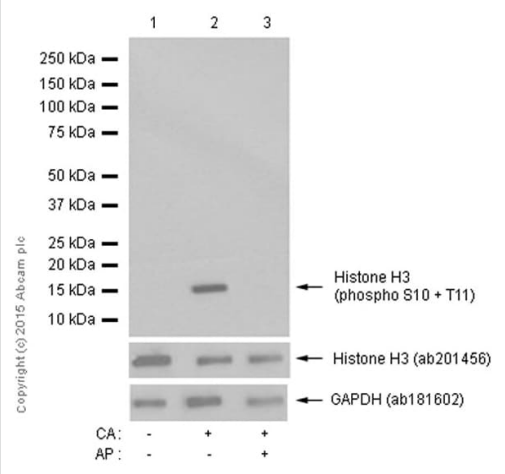
Developmental stage Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

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



Western blot - Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)

All lanes : Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107) at 1/10000 dilution

Lane 1 : Untreated HeLa cell lysate
Lane 2 : HeLa cell lysate treated with calyculin A
Lane 3 : HeLa cell lysate treated with calyculin A and alkaline phosphatase

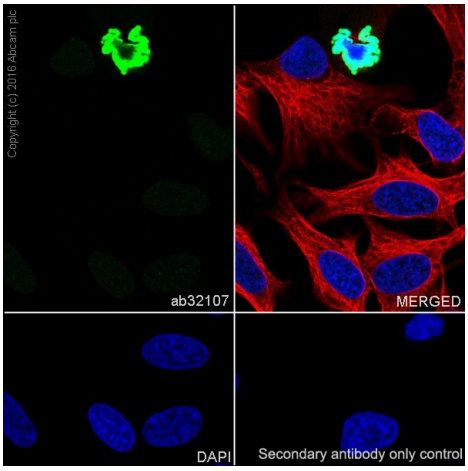
Lysates/proteins at 10 µg per lane.

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

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

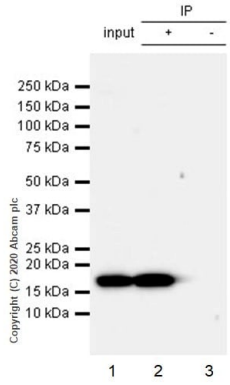
Exposure time: 1 second

Blocking and dilution buffer: 5% NFDM/TBST.



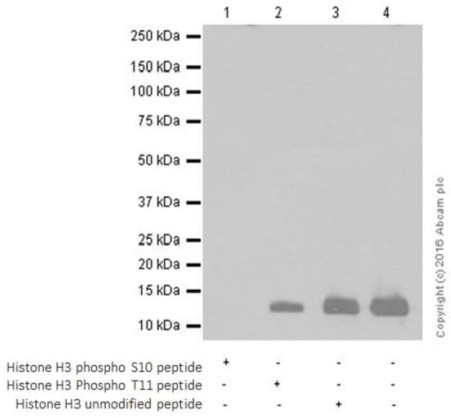
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) labeling Histone H3 (phospho S10 + T11) with ab32107 at a dilution of 1/500. Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000, 2 µg/mL) was used as the secondary antibody. Cells were counter-stained with **ab195889** Anti-Alpha Tubulin antibody [DM1A] (1/200, 2.5 µg/mL) - Microtubule Marker (Alexa Fluor® 594). DAPI (blue) was used as a nuclear counterstain.
Confocal image showing nuclear staining on mitotic HeLa cell.



Immunoprecipitation - Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)

Purified ab32107 at 1/50 dilution (2µg) immunoprecipitating Histone H3 in HeLa treated with calyculin A whole cell lysate.
Lane 1 (input): HeLa (Human cervix adenocarcinoma epithelial cell) treated with calyculin A whole cell lysate 10µg
Lane 2 (+): ab32107 + HeLa treated with calyculin A whole cell lysate.
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab32107 in HeLa treated with calyculin A whole cell lysate.
VeriBlot for IP Detection Reagent (HRP) (**ab131366**) (1/1000 dilution) was used for Western blotting.
Blocking Buffer and concentration: 5% NFDM/TBST.
Diluting buffer and concentration: 5% NFDM/TBST.
Observed band size: 17 kDa



Western blot - Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)

All lanes : Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107) at 1/1000 dilution

Lane 1 : HeLa whole cell lysate -treated with Calyculin A with Histone H3 phospho S10 peptide
Lane 2 : HeLa whole cell lysate -treated with Calyculin A with Histone H3 phospho T11 peptide
Lane 3 : HeLa whole cell lysate -treated with Calyculin A with unmodified Histone H3 peptide
Lane 4 : HeLa whole cell lysate -treated with Calyculin A with no peptide

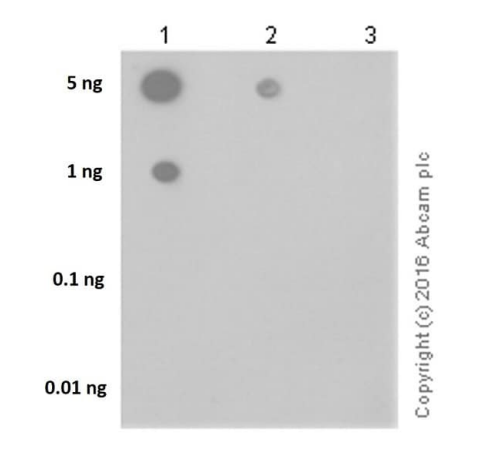
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000

dilution

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

Exposure time: 1 second

Blocking/Diluting buffer 5% NFDM/TBST

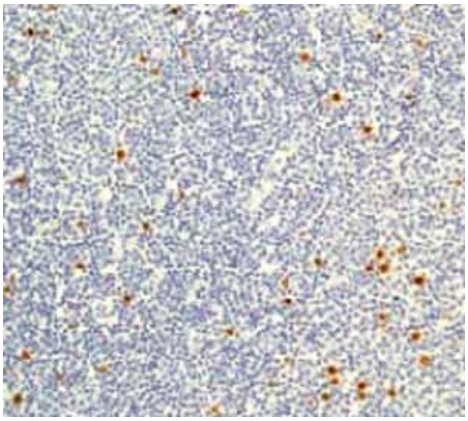


Dot Blot - Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)

Dot blot analysis of Histone H3 phospho S10 peptide (Lane 1), Histone H3 phospho T11 peptide (Lane 2) and Histone H3 unmodified peptide (Lane 3) labelling Histone H3 (phospho S10 + T11) with ab32107 at a dilution of 1/1000. **ab97051** (Peroxidase conjugated goat anti-rabbit IgG) (H+L) was used as the secondary antibody at a dilution of 1/10 000.

Blocking and diluting buffer: 5% NFDM/TBST.

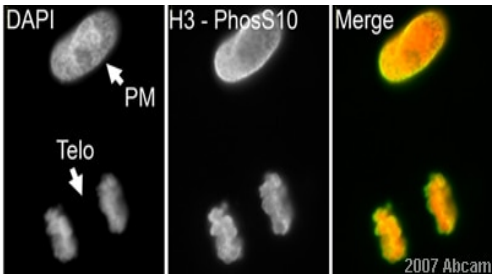
Exposure time: 3 minutes.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)

Ab32107, at a 1/50 dilution, staining Histone H3 (phospho S10 + T11) in paraffin embedded lymphoma tissue by Immunohistochemistry.

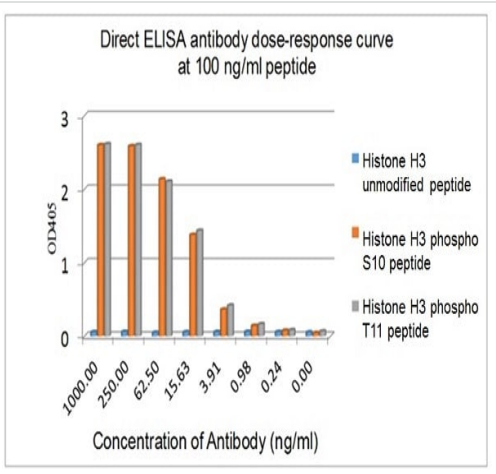
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)

This image is courtesy of an Abreview submitted by Dr Kirk McManus

ab32107 (1/1000) staining Histone H3 (phospho S10 + T11) in paraformaldehyde-fixed, DAPI counterstained HeLa cells. Secondary antibody: Goat anti-Rabbit IgG conjugated to Cy3® (1/200). Please refer to abreview for further details.

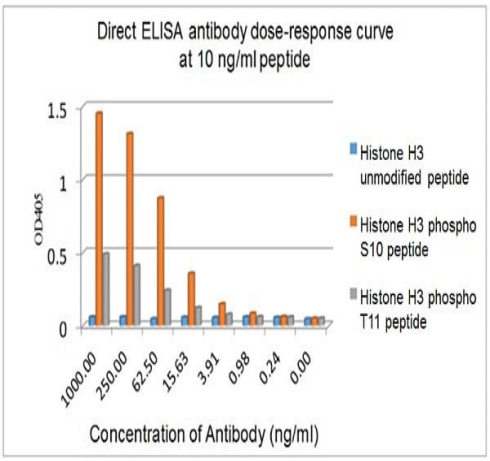


ELISA - Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)

Direct ELISA antibody dose-response curve using ab32107 (0-1000 ng/mL).

Peptides - Histone H3 unmodified peptide, Histone H3 phospho S10 peptide, Histone H3 phospho T11 peptide (100 ng/mL).

Secondary antibody - Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) (1/2500).



ELISA - Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)

Direct ELISA antibody dose-response curve using ab32107 (0-1000 ng/mL).

Peptides - Histone H3 unmodified peptide, Histone H3 phospho S10 peptide, Histone H3 phospho T11 peptide (10 ng/mL).

Secondary antibody - Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) (1/2500).

Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours

- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors