# abcam

# Product datasheet

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

יעלאעבע RabMAb

#### ★★★★ 4 Abreviews 49 References 画像数 10

## 製品の概要

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

製品の詳細 Rabbit monoclonal [E173] to Histone H3 (phospho S10 + T11)

由来種 Rabbit

特異性 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.

適用あり: ELISA, Dot blot, ICC/IF, WB, IHC-P, IP アプリケーション

適用なし: Flow Cyt

種交差性 交差種: Mouse, Human

交差が予測される動物種: Rat, Drosophila melanogaster, a wide range of other species

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 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.

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  $\mathsf{RabMAb}^{\texttt{®}}$  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.

Avoid freeze / thaw cycle.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

精製度 Protein A purified

**ポリ/モノ** モノクローナル

**クローン名** E173

アイソタイプ IgG

#### アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab32107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
ELISA		Use at an assay dependent concentration.
Dot blot		1/1000.
ICC/IF	**** (1)	1/250 - 1/1000.
WB	**** <u>(1)</u>	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.

追加情報

Is unsuitable for Flow Cyt.

#### ターゲット情報

機能

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.

配列類似性

Belongs to the histone H3 family.

発生段階

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

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 PAD4 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.

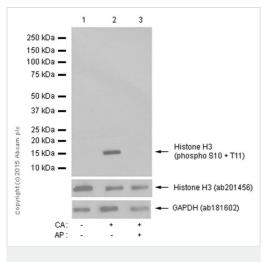
Methylation at Lys-5 (H3K4me), Lys-37 (H3K36me) and Lys-80 (H3K79me) are linked to gene activation. Methylation at Lys-5 (H3K4me) facilitates subsequent acetylation of H3 and H4. Methylation at Lys-80 (H3K79me) is associated with DNA double-strand break (DSB) responses and is a specific target for TP53BP1. 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.

細胞内局在 Nucleus. Chromosome.

画像



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.

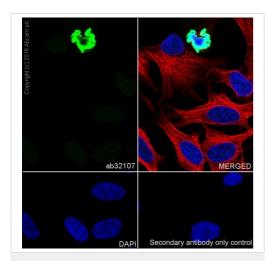
# **Secondary**

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) 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.



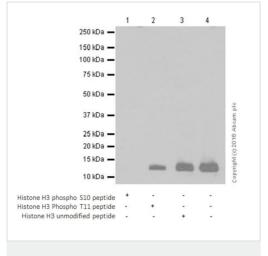
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)

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. <a href="mailto:ab150077">ab150077</a>, Alexa Fluor<sup>®</sup> 488-conjugated goat anti-rabbit IgG (1/1000, 2 µg/mL) was used as the secondary antibody. Cells were counter-stained with <a href="mailto:ab195889">ab195889</a> Anti-Alpha Tubulin antibody [DM1A] (1/200, 2.5 µg/mL) - Microtubule Marker (Alexa Fluor<sup>®</sup> 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)



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

Purified ab32107 at 1/50 dilution (2 $\mu$ g) 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 10 $\mu$ g Lane 2 (+): ab32107 + HeLa treated with calyculin A whole cell lysate.

Lane 3 (-): Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab32107 in HeLa treated with calyculin A whole cell lysate.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) (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

**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

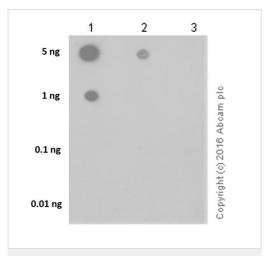
Lane 4: HeLa whole cell lysate -treated with Calyculin A with no peptide

### Secondary

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

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

Exposure time: 1 second

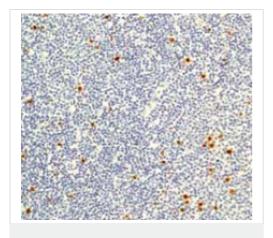


Dot Blot - Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)

Dot blot analysis of Histone H3 phospho \$10 peptide (Lane 1), Histone H3 phospho T11 peptide (Lane 2) and Histone H3 unmodified peptide (Lane 3) labelling Histone H3 (phospho \$10 + 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 1/10000.

Blocking and diluting buffer: 5% NFDM/TBST.

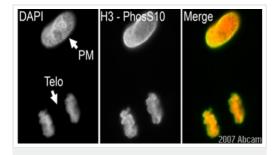
Exposure time: 3 minutes.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

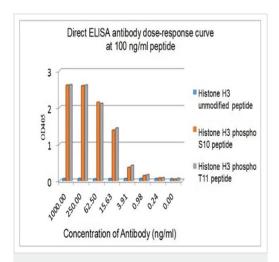
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



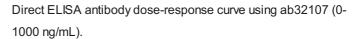
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)

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.

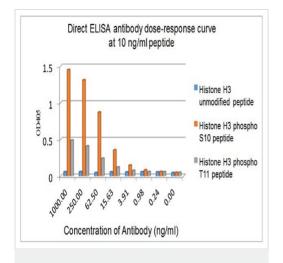


ELISA - Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)



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).

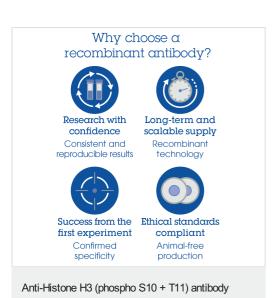


ELISA - Anti-Histone H3 (phospho S10 + T11) antibody [E173] (ab32107)

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).



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[E173] (ab32107)

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