

Product datasheet

Anti-Histone H3 (ubiquityl K23) antibody [EPR18876] ab194243

Recombinant RabMAb

5 Images

Overview

Product name	Anti-Histone H3 (ubiquityl K23) antibody [EPR18876]
Description	Rabbit monoclonal [EPR18876] to Histone H3 (ubiquityl K23)
Host species	Rabbit
Tested applications	Suitable for: Dot blot, IP, WB
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide within Human Histone H3 aa 1-100 (ubiquityl K23). The exact sequence is proprietary. Database link: P68431
Positive control	WB: Lysate of HeLa cells treated with 2mM thymidine for 18 hrs, followed by addition of fresh culture medium and incubation for 9 hrs, then treated with 2mM thymidine for 15 hrs, followed by addition of fresh culture medium and incubation for 4 hrs; Jurkat, HEK-293 lysates. IP: Lysate of HeLa cells treated with 2mM thymidine for 18 hrs, followed by addition of fresh culture medium and incubation for 9 hrs, then 2mM thymidine for 15 hrs, followed by addition of fresh culture medium and incubation for 4 hrs.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p> <p>Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough.</p> <p>Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.</p> <p>We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise[™] guarantee.</p>

In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.

We are also updating the applications & species that this product has been “predicted to work with,” however this information is not covered by our Abpromise guarantee.

Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee.

Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, as well as customer reviews and Q&As.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18876
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab194243** in the following tested applications.

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

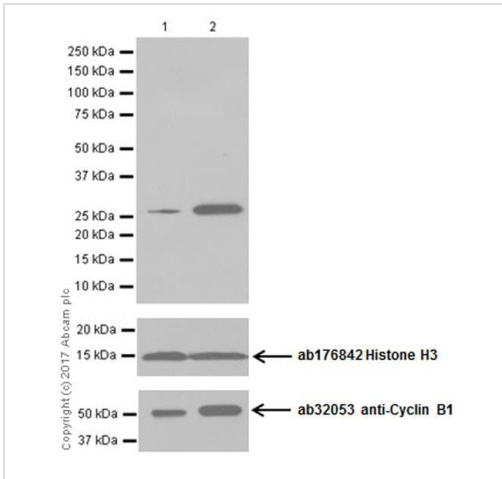
Application	Abreviews	Notes
Dot blot		Use at an assay dependent concentration.
IP		1/30.
WB		1/1000. Detects a band of approximately 28,35 kDa (predicted molecular weight: 15 kDa).

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.
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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	<p>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).</p> <p>Citrullination at Arg-9 (H3R8ci) and/or Arg-18 (H3R17ci) by PAD4 impairs methylation and represses transcription.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>Phosphorylation at Tyr-42 (H3Y41ph) by JAK2 promotes exclusion of CBX5 (HP1 alpha) from chromatin.</p> <p>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.</p>
Cellular localization	Nucleus. Chromosome.

Images



Western blot - Anti-Histone H3 (ubiquityl K23) antibody [EPR18876] (ab194243)

All lanes : Anti-Histone H3 (ubiquityl K23) antibody [EPR18876] (ab194243) at 1/20000 dilution

Lane 1 : Untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2 : Whole cell lysates from HeLa treated with 2mM thymidine for 18 hours, followed by addition of fresh culture medium and incubation for 9 hours. Cell were then treated with 2mM thymidine for 15 hours, followed by addition of fresh culture medium and incubation for 4 hours

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 15 kDa

Observed band size: 28 kDa

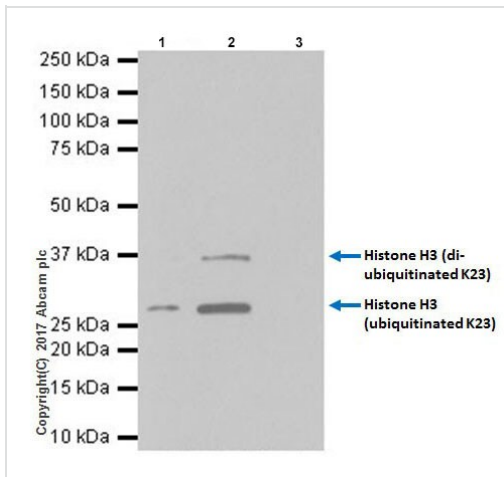
[why is the actual band size different from the predicted?](#)

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression level of H3K23ubi is increased during S phase. PMID 24013172.

The MW is consistent with literature: PMID 24013172.



Immunoprecipitation - Anti-Histone H3 (ubiquityl K23) antibody [EPR18876] (ab194243)

Histone H3 (ubiquityl K23) was immunoprecipitated using ab194243 at 1/30 dilution from 0.35 mg of whole cell lysate from HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with 2mM thymidine for 18 hours, followed by addition of fresh culture medium and incubation for 9 hours, then treated with 2mM thymidine for 15 hours, followed by addition of fresh culture medium and incubation for 4 hours.

Western blot was performed from the immunoprecipitate using ab194243 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

Lane 1: 10 µg of whole cell lysate from HeLa treated with 2mM thymidine for 18 hours, followed by addition of fresh culture medium and incubation for 9 hours, cells were then treated with 2mM thymidine for 15 hours, followed by addition of fresh culture medium and incubation for 4 hours (Input).

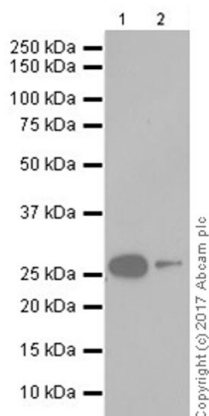
Lane 2: ab194243 IP in whole cell lysate from HeLa treated with 2mM thymidine for 18 hours, followed by addition of fresh culture medium and incubation for 9 hours, cells were then treated with 2mM thymidine for 15 hours, followed by addition of fresh culture medium and incubation for 4 hours.

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab194243 in whole cell lysate from HeLa treated with 2mM thymidine for 18 hours, followed by addition of fresh culture medium and incubation for 9 hours, cells were then treated with 2mM thymidine for 15 hours, followed by addition of fresh culture medium and incubation for 4 hours.

Blocking and dilution buffer and concentration: 5% NFDm/TBST.

Exposure time: 3 seconds.

The band at around 35kDa can be explained as di-ubiquitinated K23 (PMD24013172).



Western blot - Anti-Histone H3 (ubiquityl K23) antibody [EPR18876] (ab194243)

All lanes : Anti-Histone H3 (ubiquityl K23) antibody [EPR18876] (ab194243) at 1/20000 dilution

Lane 1 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate at 20 µg

Lane 2 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

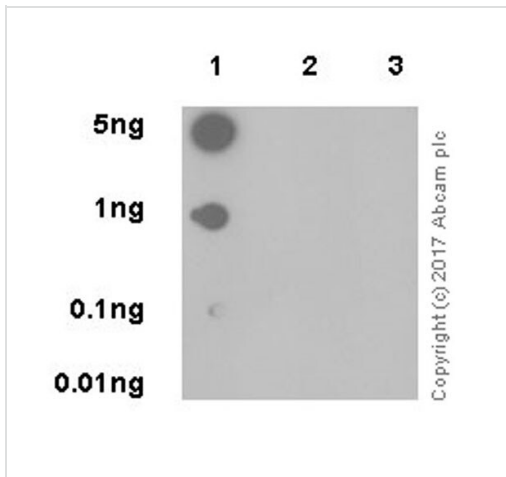
Predicted band size: 15 kDa

Observed band size: 28 kDa [why is the actual band size different from the predicted?](#)

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDm/TBST.

The MW is consistent with literature: PMID 24013172.



Dot blot analysis of Histone H3 (ubiquityl K23) labeled with ab194243 at 1/1000 dilution.

Lane 1: Histone H3 (ubiquitinated K23) peptide (aa20-29).

Lane 2: Histone H3 non-ubiquitinated peptide (aa22-31).

Lane 3: Histone H3 full length recombinant protein.

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 was used as secondary antibody.

Blocking and diluting buffer: 5% NFDN/TBST.

Exposure time: 3 minutes.

Dot Blot - Anti-Histone H3 (ubiquityl K23) antibody [EPR18876] (ab194243)

Why choose a recombinant antibody?

- Research with confidence**
Consistent and reproducible results
- Long-term and scalable supply**
Recombinant technology
- Success from the first experiment**
Confirmed specificity
- Ethical standards compliant**
Animal-free production

Anti-Histone H3 (ubiquityl K23) antibody [EPR18876] (ab194243)

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

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