abcam

Product datasheet

Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade ab1220

72 Abreviews 886 References 11 Images

Overview

Product name	Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade
Description	Mouse monoclonal [mAbcam 1220] to Histone H3 (di methyl K9) - ChIP Grade
Host species	Mouse
Specificity	By peptide ELISA ab1220 recognizes di methyl K9, but not unmodified K9, mono methyl K9, tri methyl K9, di methyl K27, tri methyl K27, mono methyl K4, di methyl K4 or tri methyl K4. By Western blot ab1220 is blocked by di methyl K9, but not by unmodified K9, mono methyl K9, tri methyl K9, di methyl K27, tri methyl K27, mono methyl K4, di methyl K4 or tri methyl K4. This indicates the specificity of ab1220 for di methyl K9 of Histone H3.
Tested applications	**Suitable for:** ICC/IF, WB, ELISA, IHC-P, ChIP
Species reactivity	**Reacts with:** Cow, Human, Arabidopsis thaliana, Drosophila melanogaster, Rice **Predicted to work with:** Mouse, Rat, Sheep, Chicken, Saccharomyces cerevisiae, Xenopus laevis, Caenorhabditis elegans, Schizosaccharomyces pombe, Corn, Common marmoset, Other species
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab1772**)
Positive control	IHC-P: Human kidney tissue; Mouse liver tissue. WB: HeLa whole cell lysate; Calf thymus histone lysate; HEK-293 lysate. ChIP: AFM ChIP primer pair **ab269259**; Chromatin prepared from U2OS cells. ICC/IF: HeLa cells.
General notes	Learn about ChIP assay kits, other ChIP antibodies, and more in the **ChIP assay guide**. **ChIP protocols:** **ChIP protocol for cross-linking ChIP (X-ChIP)** **Native ChIP protocol** **Chromatin preparation from tissues for ChIP** **ChIP troubleshooting** **ChIP tips and tricks guide** This antibody clone [mAbcam 1220] is manufactured by Abcam. If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact **orders@abcam.com** or you can find further information **here**. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. 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, along with publications, 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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Purity	Protein G purified
Clonality	Monoclonal
Clone number	mAbcam 1220
Isotype	IgG2a
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab1220 in the following tested applications.

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

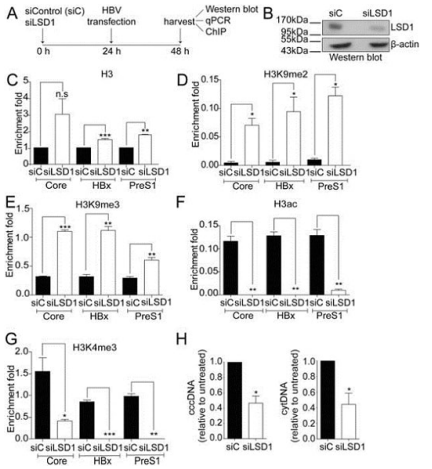
Application	Abreviews	Notes
ICC/IF	★★★★☆ **(16)**	Use a concentration of 5 µg/ml.
WB	★★★★★ **(33)**	Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 17 kDa).Can be blocked with **Human Histone H3 (di methyl K9) peptide (ab1772)**.
ELISA		Use a concentration of 0.00025 - 1 µg/ml. when testing with immunogen peptide.
IHC-P	★★★★★ **(7)**	Use at an assay dependent concentration.
ChIP	★★★★★ **(11)**	Use 2-4 µg for 25 µg of chromatin. Use AFM ChIP primer pair **ab269259** as positive control.

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.

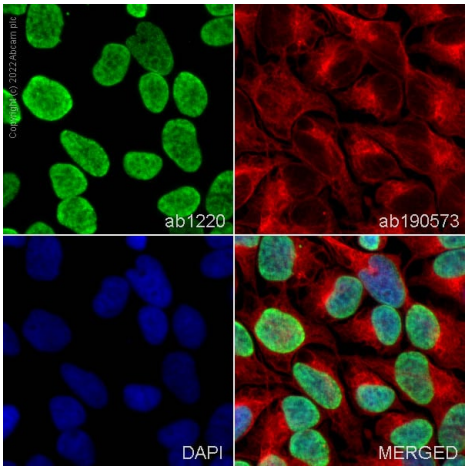
Images



ChIP - Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220)

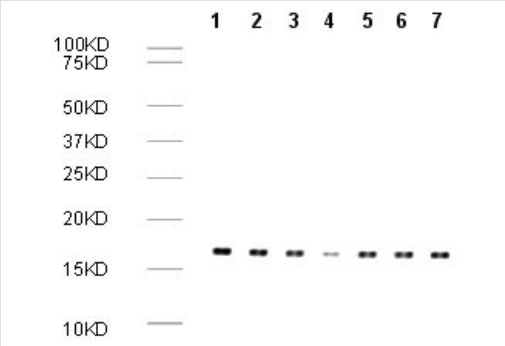
Image from Alarcon V et al., Sci Rep 13(6), Fig 4. Doi: 10.1038/srep25901. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Alarcon V et al investigates the effects of pargyline due to inhibition of LSD1. Levels of LSD1 protein were reduced using siRNA (siLSD1). Huh7 (Human hepatocellular carcinoma) cells were treated with and without siLSD1 and incubated for 24 hours before transfection of HBV genome (A). Western blot analysis shows the reduction of LSD1 after treatment. Covalent post-translational modifications on Histone H3 was determined by ChIP using ab1220 as one of the specific antibodies (C-G). HBV cccDNA and cytoplasmic levels were determined by qPCR (H).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220)

ab1220 staining Histone H3 (di methyl K9) in HeLa (Human cervix adenocarcinoma epithelial cell) cells. The cells were fixed with 4% PFA (10 mins), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab1220 at 5 µg/ml and **ab190573**, Rabbit monoclonal to alpha Tubulin (Alexa Fluor® 647), at 2 µg/ml (shown in red). The secondary antibody (shown in green) was **ab150117**, Alexa Fluor® 488 Goat anti-Mouse IgG (H+L) used at a 1/1000 dilution for 1h at room temperature. Nuclear DNA was labelled with DAPI (shown in blue).
Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.
This product also gave a positive outcome under the same testing conditions in HeLa cells fixed with 100% methanol (5 mins).



Western blot - Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220)

All lanes : Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220)

Lane 1 : Calf thymus histone lysate
Lane 2 : Calf thymus histone lysate with Histone H3 peptide - unmodified at 1 µg/ml
Lane 3 : Calf thymus histone lysate with Human Histone H3 (mono methyl K9) peptide (**ab1771**) at 1 µg/ml
Lane 4 : Calf thymus histone lysate with Human Histone H3 (di methyl K9) peptide (**ab1772**) at 1 µg/ml
Lane 5 : Calf thymus histone lysate with Human Histone H3 (tri methyl K9) peptide (**ab1773**) at 1 µg/ml
Lane 6 : Calf thymus histone lysate with Human Histone H3 (di methyl K4) peptide (**ab7768**) at 1 µg/ml
Lane 7 : Calf thymus histone lysate with Human Histone H3 (di methyl K27) peptide (**ab1781**) at 1 µg/ml

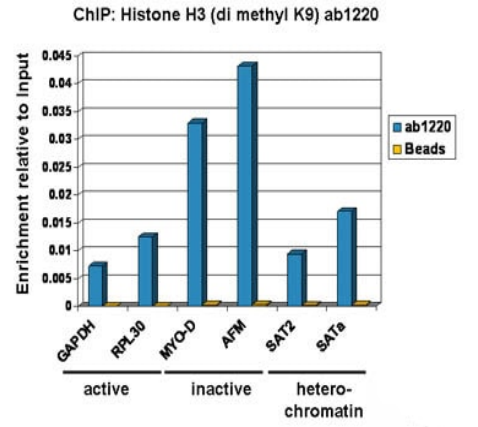
Secondary
All lanes : Rabbit Anti-Mouse IgG H&L (HRP) (**ab6728**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

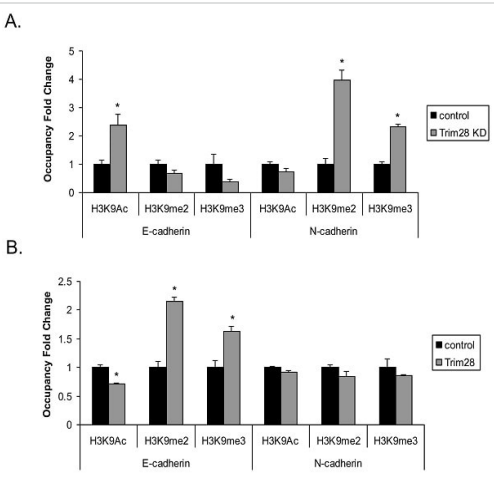
Predicted band size: 17 kDa

Exposure time: 1 minute



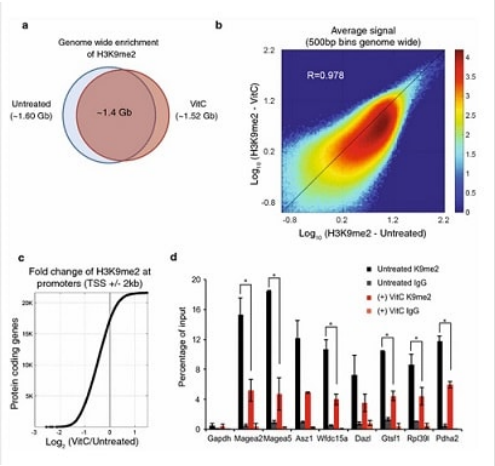
ChIP - Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220)

Chromatin was prepared from U2OS cells according to the **Abcam X-ChIP protocol**. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25µg of chromatin, 2µg of ab1220 (blue), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are located in the first Kb of the transcribed region.



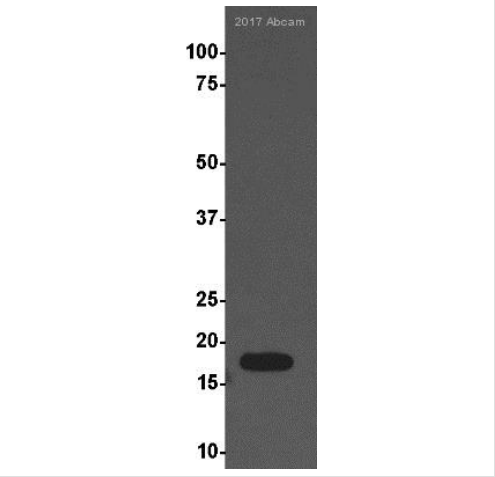
ChIP - Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220)

Chen L et al investigates role of Trim 28 (Tripartite motif containing 28) in the epithelial to mesenchymal transition which is implicated in cancer metastasis. ChIP was performed using ab1220. ChIP assay was performed in Trim 28 knockdown A549 cells and control (A). ChIP assay was performed in control and Trim28 expressed in A549 cells (B).



ChIP - Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220)

ChIP was performed using ab1220. Naïve Mouse ES cells were resuspended in PBS and crosslinked with 1% formaldehyde for 5 minutes at room temperature. Crosslinking was quenched with 125m M glycine. A mouse anti IgG (**ab18413**) was used as a control. ChIP -seq for H3K9me2 in ES cells treated with and without vitamin C (image a). H3K9me2 signal genome-wide was plotted comparing untreated and vitamin C-treated cells (image b). Fold change for average H3K9me2 signal at gene promoters. Most gene promoters display a reduction in H3K9me2 in vitamin C-treated ES cells (image c). ChIP-qPCR for H3K9me2 in ES cells with or without vitamin C at gene promoters. ChIP for IgG was performed as a negative control (image d). Asterisks represent P < 0.05 by t test



Western blot - Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220)

This image is courtesy of an anonymous abreview.

Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220) at 1/1000 dilution + HEK293 at 30 µg

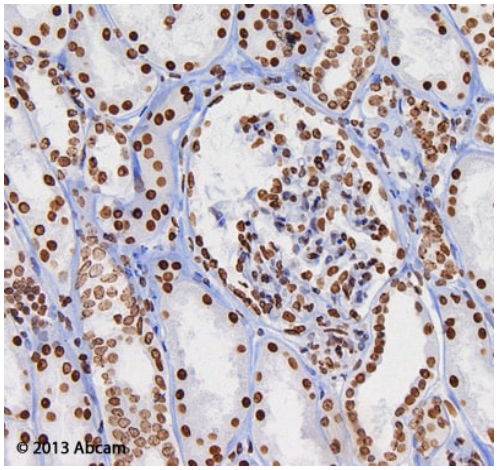
Secondary

HRP conjugated polyclonal Sheep anti-Mouse IgG at 1/5000 dilution

Performed under reducing conditions.

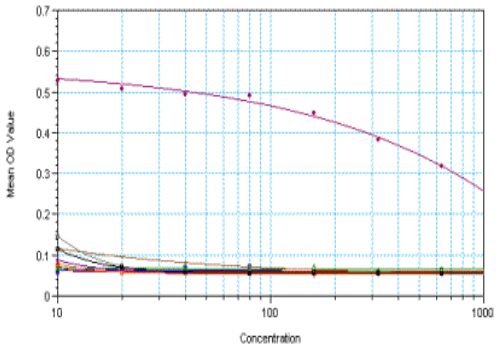
Predicted band size: 17 kDa

Blocking buffer: 5% milk



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220)

ab1220 staining human kidney sections by IHC-P using EXPOSE IHC detection kit (**ab80436**). Formalin fixed paraffin embedded tissue sections were pre-treated using heat mediated antigen retrieval (using a pressure cooker) with sodium citrate buffer (pH6) for 30 mins. The section was incubated with ab1220, 5µg/ml, for 1 hour at room temperature. DAB was used as the chromogen and the section was counterstained with haematoxylin and mounted with DPX.

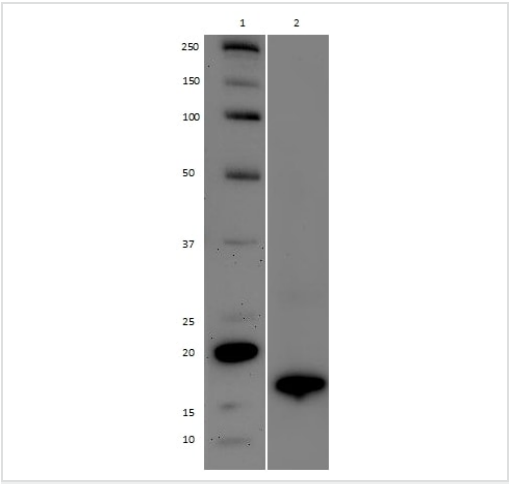


ELISA - Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220)

ELISA using ab1220 at varying antibody concentrations.

The purple line indicates binding to the Human Histone H3 (di methyl K9) peptide **ab1772**. Binding to the following peptides was not seen:
Human Histone H3 (unmodified) peptide (**ab2903**),
Human Histone H3 (mono methyl K9) peptide (**ab1771**),
Human Histone H3 (tri methyl K9) peptide (**ab1773**),
Human Histone H3 (di methyl K27) peptide (**ab1781**),
Human Histone H3 (tri methyl K27) peptide (**ab1782**),
Human Histone H3 (mono methyl K4) peptide (**ab1340**),
Human Histone H3 (di methyl K4) peptide (**ab7768**),
Human Histone H3 (tri methyl K4) peptide (**ab1342**).

This indicates the specificity of ab1220 for di methyl K9 of Histone H3.



Western blot - Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220)

Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220) at 1 µg/ml + HeLa (Human epitherlial carcinoma cell line) Whole Cell Lysate at 20 µg

Secondary
Goat polyclonal to Mouse IgG-H&L- Pre-Adsorbed (HRP) at 1/10000 dilution

Developed using the ECL technique.

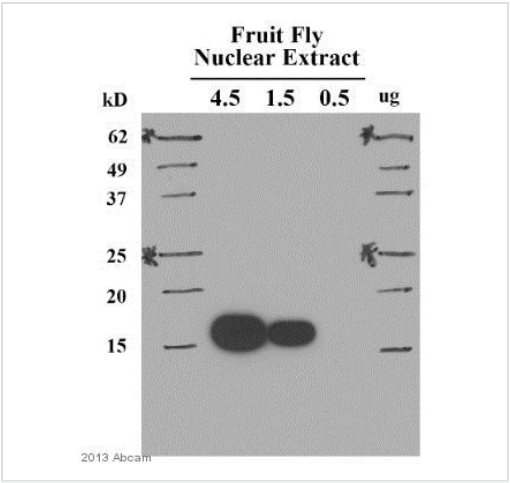
Performed under reducing conditions.

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

Exposure time: 5 minutes

Lane 1 : Marker.

All blocking and antibody incubation steps were done with 5% milk in 20mM Tris-HCL, and 0.1% TWEEN-20.



Western blot - Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220)
This image is courtesy of an anonymous Abreview

All lanes : Anti-Histone H3 (di methyl K9) antibody [mAbcam 1220] - ChIP Grade (ab1220) at 2.5 µg/ml

Lane 1 : Fruit fly embryo tissue lysate - nuclear at 4.5 µg
Lane 2 : Fruit fly embryo tissue lysate - nuclear at 1.5 µg
Lane 3 : Fruit fly embryo tissue lysate - nuclear at 0.5 µg

Secondary
All lanes : HRP-conjugated Goat anti-mouse IgG polyclonal at 1/2000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 17 kDa

Observed band size: 15 kDa

Exposure time: 1 minute

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