abcam

ab6000

Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade

Key facts

Isotype	IgG2b
Host species	Mouse
Storage buffer	pH: 7.4 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	mAbcam 6000
Purification technique	Affinity purification
Specificity	This antibody detects a band of the appropriate size when used in Western blotting on a calf thymus histone preparation (see image). It is specifically blocked by peptides corresponding to di methylated K4 of Histone H3 and to tri methylated K4. Slight cross reactivity is observed with mono methyl K4 peptide in ELISA and Western Blot.
Light chain type	kappa
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ChIP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Cow, Rice
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Mouse, Rat, Rabbit, Chicken, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe, Neurospora crassa, Candida albicans
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1 µg for 10^6 Cells
Notes	ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.

Expected

Species	Cow
Dilution info	-
Notes	-

Species	Rice
Dilution info	-

Notes	ab170192 - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.

Predicted

Species	Mouse, Rat, Rabbit, Chicken, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe, Neurospora crassa, Candida albicans
Dilution info	-
Notes	-

WB

Tested

Species	Cow
Dilution info	1/500
Notes	-

Expected

Species	Human
Dilution info	-
Notes	-

Species	Rice
Dilution info	1/500
Notes	-

Predicted

Species	Mouse, Rat, Rabbit, Chicken, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe, Neurospora crassa, Candida albicans
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1 µg/mL
Notes	-

Expected

Species	Cow
Dilution info	-
Notes	-

Species	Rice
Dilution info	1 µg/mL
Notes	-

Predicted

Species	Mouse, Rat, Rabbit, Chicken, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe, Neurospora crassa, Candida albicans
Dilution info	-
Notes	-

Target data

See full target information H3C1 tri methyl K4 + di methyl K4

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.

Storage

Shipped at conditions Blue Ice

Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Want a custom formulation?
This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

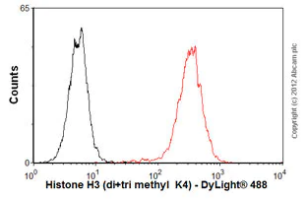
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

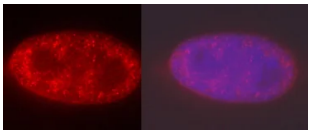
5 product images



Flow Cytometry (Intracellular) - Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade (ab6000)

Histone H3 (di methyl K4, tri methyl K4) Flow Cytometry (Intracellular) staining using mouse Anti-Histone H3 (di methyl K4, tri methyl K4) antibody

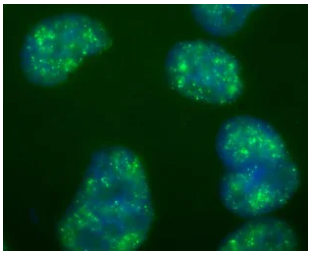
Overlay histogram showing HeLa cells stained with ab6000 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab6000, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2b [PLPV219] (ab91366, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade (ab6000)

Histone H3 (di methyl K4, tri methyl K4) Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Histone H3 (di methyl K4, tri methyl K4) antibody

Left image shows human Hep cell monolayer fixed with 4% formaldehyde, permeabilized using 0.5% Triton X-100, blocked with 5% FCS in PBS (20 min) and incubated with ab6000 for 1 hr (diluted 1/200 in PBS plus 1% FCS). Detection with anti-mouse rhodamin labeled secondary antibody. Unstained areas represent nucleoli and heterochromatin as judged from DAPI overlay of the same cell in the right image.
This image was kindly supplied as part of the review submitted by Christian Schoefer.



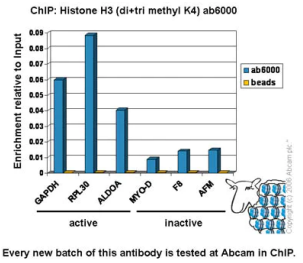
This image is courtesy of Luke Hughes-Davies and Rhiannon Jade, Gurdon Institute, Cambridge, UK

Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade (ab6000)

Histone H3 (di methyl K4, tri methyl K4) Immunocytochemistry/ Immunofluorescence staining using mouse Anti-Histone H3 (di methyl K4, tri methyl K4) antibody

Immunofluorescent imaging of human cells (U2OS) with ab6000 reveals a diffuse background nuclear staining corresponding to global dimethylation of K4, with multiple foci of brighter staining. The complete lack of nucleolar or cytoplasmic staining background confirms the high specificity of this antibody in this application.
IF was performed with a standard paraformaldehyde technique (fixed in PBS buffered PFH 4% for 5 minutes, permeabilised with 0.5% triton-PBS for 5 minutes, blocked with 5% milk / 0.2% tween for one hour. Primary antibody used at 1/200 in 5% milk / 0.2% TWEEN for one hour, secondary antibody Alexa 488 for 30 minutes. All blocking and incubation steps carried out at 37 degrees.

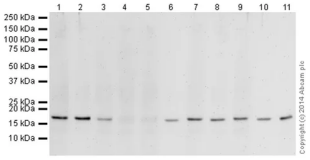
Due to the size constraints for images on our website, unfortunately we are unable to show a higher quality image.



Every new batch of this antibody is tested at Abcam in ChIP.

ChIP - Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade (ab6000)

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 25 µg of chromatin, 2 µg of ab6000 (blue), and 20 µl 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). Primers and probes are located in the first kb of the transcribed region.



Western blot - Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade (ab6000)

Histone H3 (di methyl K4, tri methyl K4) Western blot staining of Calf Thymus Histone Preparation Nuclear Lysate using mouse Anti-Histone H3 (di methyl K4, tri methyl K4) antibody

All lanes:
Western blot - Anti-Histone H3 (di methyl K4, tri methyl K4) antibody [mAbcam 6000] - ChIP Grade (ab6000) at 1 µg/mL

Lane 1:
Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg

Lane 2:
Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg with Human Histone H3 (unmodified) peptide (ab7228)

Lane 3:
Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg with Human Histone H3 (mono methyl K4) peptide (ab1340)

Lane 4:
Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg with Human Histone H3 (di methyl K4) peptide (ab7768)

Lane 5:
Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg with Human Histone H3 (tri methyl K4) peptide (ab1342)

Lane 6:
Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg with Human Histone H3 (mono methyl K9) peptide (ab1771)

Lane 7:
Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg with Human Histone H3 (di methyl K9) peptide (ab1772)

Lane 8:
Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg with Human Histone H3 (tri methyl K9) peptide (ab1773)

Lane 9:
Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg with Human Histone H3 (mono methyl K27) peptide (ab1780)

Lane 10:
Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg with Human Histone H3 (di methyl K27) peptide (ab1781)

Lane 11:

Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg with Human Histone H3 (tri methyl K27) peptide (ab1782)

Secondary

All lanes:
Western blot - Goat Anti-Mouse IgG H&L (HRP) preadsorbed (ab97040) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa

Observed band size: 17 kDa

Exposure time: 20min

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.