abcam

ab32356

# Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade

Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356) is a rabbit monoclonal antibody detecting Histone H3 in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF, ChIP, CUT&Tag Seq**. Suitable for **African green monkey, Chicken, Cow, Human, Mouse, Rat**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 200 publications
- Trusted since 2006

Recombinant | RabMAb | Advanced Validation | 20ul selling size

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Clone number** | Y47 |
| **Purification technique** | Affinity purification Protein A |
| **Concentration** | 0.586 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

## CUT&Tag-seq

**Tested**

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/100 |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Human, Rat, African green monkey, Chicken, Cow |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Sheep, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Monkey, Zebrafish, Mammals, Rice |
| **Dilution info** | - |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## PepArr

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human, Mouse, Rat, Sheep, Chicken, Cow, Human, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Monkey, Zebrafish, Mammals, African green monkey, Rice |
| **Dilution info** | - |
| **Notes** | - |

## ChIC/CUT&RUN-seq

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat, African green monkey, Chicken, Cow |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Sheep, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Monkey, Zebrafish, Mammals, Rice |
| **Dilution info** | - |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## IP

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/30 |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat, African green monkey, Chicken, Cow |
| **Dilution info** | Use at an assay dependent concentration. |

| | |
|---|---|
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Sheep, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Monkey, Zebrafish, Mammals, Rice |
| **Dilution info** | - |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## ChIP

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 2 µg for 25 µg chromatin |
| **Notes** | Use ALDOA ChIP primer pair ab269260 as positive control. |

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat, African green monkey, Chicken, Cow |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Sheep, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Monkey, Zebrafish, Mammals, Rice |
| **Dilution info** | - |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/2000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | 1/2000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | African green monkey |
| **Dilution info** | 1/2000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Chicken |
| **Dilution info** | 1/2000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/2000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Cow |
| **Dilution info** | 1/2000 |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Sheep, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Monkey, Zebrafish, Mammals, Rice |

| | |
|---|---|
| **Dilution info** | - |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/1000 |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat, African green monkey, Chicken, Cow |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Sheep, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Monkey, Zebrafish, Mammals, Rice |
| **Dilution info** | - |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## ChIP-seq

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 4 µg for 30 µg chromatin |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat, African green monkey, Chicken, Cow |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Sheep, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Monkey, Zebrafish, Mammals, Rice |
| **Dilution info** | - |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/20 - 1/100 |
| **Notes** | ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. |

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat, African green monkey, Chicken, Cow |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Sheep, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Monkey, Zebrafish, Mammals, Rice |
| **Dilution info** | - |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## IHC-P

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/800 |
| **Notes** | Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | 1/800 |
| **Notes** | Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/800 |
| **Notes** | Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |

### Expected

| | |
|---|---|
| **Species** | African green monkey, Chicken, Cow |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Sheep, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis thaliana, Monkey, Zebrafish, Mammals, Rice |
| **Dilution info** | - |
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Synthetic peptide - Human |
| **Dilution info** | - |
| **Notes** | - |

## Target data

See full target information H3C1 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** | Conditional Ambient |
| **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

**What is this antibody validated in?**
Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF), ChIP in African green monkey, Chicken, Cow, Human, Mouse, Rat samples.

**What is the molecular weight of Histone H3?**
Anti-Histone H3 (di methyl K4) [Y47] - ChIP Grade (ab32356) specifically detects a band for Histone H3 (UniProt: P68431) at a molecular weight of 15kDa.

**Trusted by the scientific community**
Anti-Histone H3 (di methyl K4) [Y47] - ChIP Grade (ab32356) was first used in a scientific publication in 2006 and has been cited over 200 times in peer-reviewed journals.

**Reviewed by scientists**
Anti-Histone H3 (di methyl K4) [Y47] - ChIP Grade (ab32356) has over 10 independent reviews from customers.

**Other related products**
We have a range of other formats of antibody clone [Y47] also available for your convenience: ab32356, Carrier free - ab173324, Alexa Fluor® 488 - ab200024, Alexa Fluor® 647 - ab200026, Alexa Fluor® 594 - ab207864, Alexa Fluor® 405 - ab207865, PE - ab208741

**Patented technology**
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

**What are the advantages of a recombinant monoclonal antibody?**
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 batch production

For more information, read more on recombinant antibodies.

**Trial sizes available!**
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

## 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.

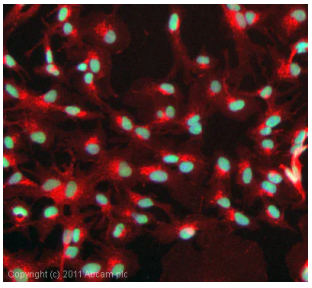
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We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

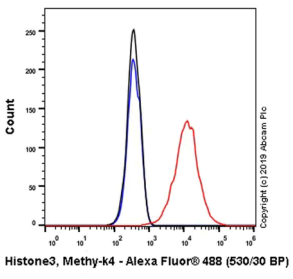
## 18 product images



### Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Histone H3 (di methyl K4) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Histone H3 (di methyl K4) antibody

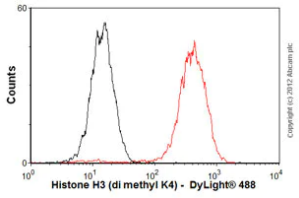
ICC/IF image of unpurified ab32356 stained HepG2 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab32356 , 5µg/ml) overnight at +4°C. The secondary antibody (green) was a goat anti-rabbit DyLight® 488 (IgG - H&L, pre-adsorbed) (ab96899) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.




### Flow Cytometry (Intracellular) - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Histone H3 (di methyl K4) Flow Cytometry (Intracellular) staining using rabbit Anti-Histone H3 (di methyl K4) antibody

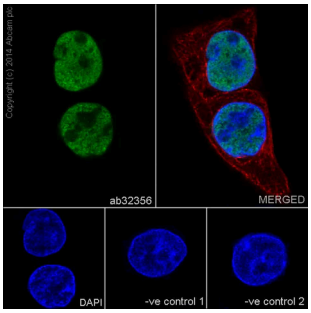
Intracellular Flow Cytometry analysis of HepG2 (human hepatocellular carcinomal) cells labeling Histone H3 di methyl K4 with purified ab32356 at 1/500 dilution (1.17µg/ml) (Red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal IgG (ab172730) / Black. Unlabeled control - Unlabelled cells / blue.




## Flow Cytometry (Intracellular) - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Histone H3 (di methyl K4) Flow Cytometry (Intracellular) staining using rabbit Anti-Histone H3 (di methyl K4) antibody

Overlay histogram showing HeLa cells stained with unpurified ab32356 (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 (ab32356, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was a goat anti-rabbit DyLight® 488 (IgG; H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x$10^6$ cells) used under the same conditions. Acquisition of >5,000 events was performed.

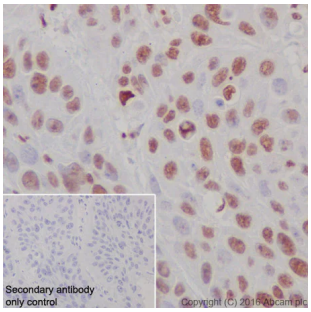



## Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Histone H3 (di methyl K4) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Histone H3 (di methyl K4) antibody

Immunocytochemistry/Immunofluorescence analysis of HepG2 cells labelling Histone H3 (di methyl K4) with purified ab32356 at a dilution of 1/1000. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. The cells were co-stained with ab7291, a mouse anti-tubulin (1/1000) using ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) as the secondary antibody. Nuclei counterstained with DAPI (blue).
Control 1: primary antibody (1/1000) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

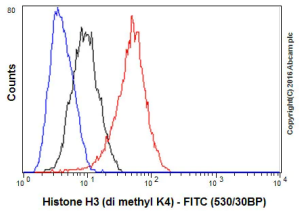
Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).




## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Histone H3 (di methyl K4) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Histone H3 (di methyl K4) antibody

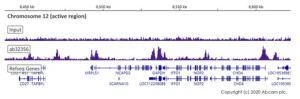
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labelling Histone H3 (di methyl K4) with purified ab32356 at a dilution of 1/800. Antigen retrieval was performed using Tris/EDTA buffer, pH9. ab97051, a HRP-conjugated goat anti-rabbit IgG H&L was used as the secondary antibody (1/500). Counter stained with hematoxylin.




## Flow Cytometry (Intracellular) - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

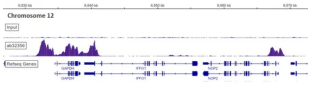
Histone H3 (di methyl K4) Flow Cytometry (Intracellular) staining using rabbit Anti-Histone H3 (di methyl K4) antibody

Intracellular Flow Cytometry analysis of HeLa cells labelling Histone H3 (di methyl K4) with purified ab32356 at 1/150 (red). Cells were fixed with 80% methanol. An Alexa Fluorr® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG (ab172730). Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



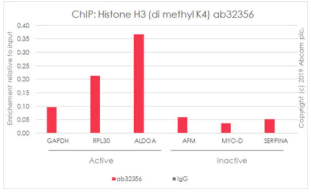
## ChIP-sequencing - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with $10^7$ cells and 4 µg of Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356). ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.
Additional screenshots of mapped reads can be downloaded here.



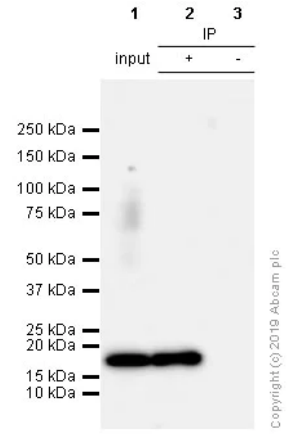
## ChIP-sequencing - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 30 µg of chromatin and 4 µg of Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356). ChIP DNA was sequenced on the Illumina NextSeq 500 to a depth of 30 million reads. ChIP-Seq validation performed by Active Motif, Carlsbad, CA.
Additional screenshots of mapped reads can be downloaded here.




## ChIP - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of ab32356 (red), and 20µl of Anti Rabbit IgG sepharose beads. 2µg of rabbit normal IgG was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach). Primers and probes are located in the first kb of the transcribed region.



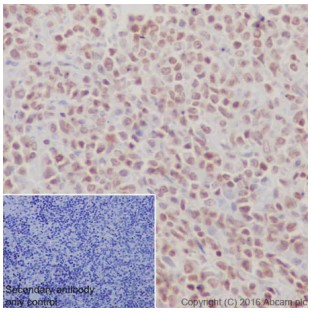

## Immunoprecipitation - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

ab32356 (purified) at 1/30 dilution (20 µg/ml) immunoprecipitating Histone H3 di methyl K4 in HepG2 whole cell lysate.

**Lane 1 (input):** HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate 10µg
**Lane 2 (+):** ab32356 & HepG2 whole cell lysate
**Lane 3 (-):** Rabbit monoclonal IgG (ab172730) instead of ab32356 in HepG2 whole cell lysate
For western blotting, ab32356 at 1/500 and veriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/1000 dilution.

Blocking and diluting buffer: 5% NFDM /TBST.
All lanes:
Immunoprecipitation - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

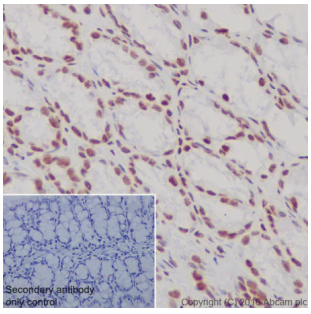
Predicted band size: 15 kDa




## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Histone H3 (di methyl K4) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Histone H3 (di methyl K4) antibody

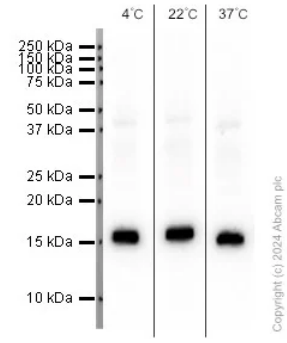
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat spleen tissue labelling Histone H3 (di methyl K4) with purified ab32356 at a dilution of 1/800. Antigen retrieval was performed using Tris/EDTA buffer, pH9. ab97051, a HRP-conjugated goat anti-rabbit IgG H&L was used as the secondary antibody (1/500). Counter stained with hematoxylin.




## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Histone H3 (di methyl K4) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Histone H3 (di methyl K4) antibody

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse colon tissue labelling Histone H3 (di methyl K4) with purified ab32356 at a dilution of 1/800. Antigen retrieval was performed using Tris/EDTA buffer, pH9. ab97051, a HRP-conjugated goat anti-rabbit IgG H&L was used as the secondary antibody (1/500). Counter stained with hematoxylin.




## Western blot - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Histone H3 (di methyl K4) Western blot staining of HT-29 whole cell lysate using rabbit Anti-Histone H3 (di methyl K4) antibody

This blot was produced using 14% SDS-PAGE containing 15 µg of HT-29 whole cell lysate per lane at 150V for 50 minutes before being transferred onto a 0.45 µm PVDF membrane at 72V for 50 minutes. The membrane was then blocked for 1hr using 5% NFDM/TBST, then incubated with ab32356 (1/10,000) at room temperature for 1hr. After being washed three times in TBST, the

membrane was incubated with Peroxidase conjugated goat anti-rabbit IgG (H+L) (ab97051) at 1/20,000 dilution for 1hr at room temperature. The membrane was washed three times again. Then the signal was developed using the ECL technique.

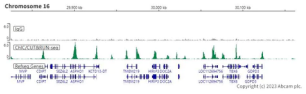
ab32356 was stored at a range of temperatures (+4°C, +22°C, +37°C) for 1 week before being tested in WB. The image shows the band intensity remains relatively constant across all storage temperatures, demonstrating that antibody activity is not affected under these conditions. This data was generated as part of a structured antibody stability study supporting Abcam’ s transition from cold⊠chain to ambient shipping.
All lanes:
Western blot - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356) at 1/10000 dilution

All lanes:
HT-29 whole cell lysate at 15 µg with NDFM/TBST

Secondary

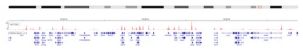
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Exposure time: 20s



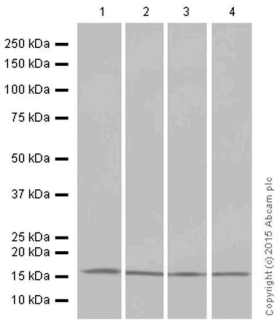
## ChIC/CUT&RUN sequencing - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and 5 µg of ab32356 [Y47]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control ab172730 is also shown. Additional screenshots of mapped reads can be found in the Protocol booklet in the Product Protocol section. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



## CUT&Tag sequencing - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

Nuclei isolated from adult mouse cortex were resuspended in antibody buffer (20 mM HEPES pH 7.5, 150 mM NaCl, 0.5 mM spermidine, 2 mM EDTA, 0.05% digitonin, 0.01% NP-40, 2% BSA, protease inhibitors). 200,000 nuclei were incubated overnight at 4 °C with 1 µl primary antibody in 100 µl antibody buffer (1:100). After two washes in high-salt Dig-300 buffer (as above, 300 mM NaCl, no EDTA), nuclei were incubated with anti-rabbit nanobody-Tn5 for 1 h at room temperature in high-salt Dig-300, washed, and tagmented in the same buffer supplemented with 10 mM $MgCl_2$ for 1 h at 37 °C. Purified DNA was amplified with indexed i5/i7 primers and sequenced paired-end on the Illumina NovaSeq X to a depth of -10 million reads. Data were kindly provided by an anonymous collaborator.




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All lanes:
Western blot - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356) at 1/10000 dilution

Lane 1:
HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

Lane 2:
HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate at 20 µg

Lane 3:
SH-SY5Y (Human neuroblastoma cell line from bone marrow) whole cell lysate at 20 µg

Lane 4:
C6 (Rat glial tumor cell line) whole cell lysate at 20 µg

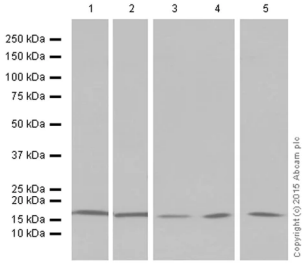
Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 15 kDa

Observed band size: 17 kDa

Exposure time: 5s




## Western blot - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

All lanes:
Western blot - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356) at 1/2000 dilution

Lane 1:
L6 (Rat skeletal muscle cell line) whole cell lysate at 20 µg

Lane 2:
NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate at 20 µg

Lane 3:
COS-1 (African green monkey kidney fibroblast-like cell line) whole cell lysate at 20 µg

Lane 4:
UMNSAH/DF-1 (Transformed chicken embryonic fibroblast cell line) whole cell lysate at 20 µg

Lane 5:
MDBK (Bovine kidney cell line) whole cell lysate at 20 µg

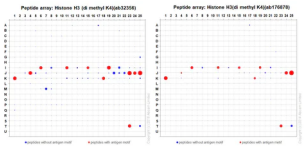
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/1000 dilution

Predicted band size: 15 kDa

Observed band size: 17 kDa

Exposure time: 5s



## Peptide Array - Anti-Histone H3 (di methyl K4) antibody [Y47] - ChIP Grade (ab32356)

ab32356 or ab176878 was tested in Peptide Array against 501 different modified and unmodified histone peptides; each peptide is printed on the array at six concentrations (each in triplicate). Circle area represents affinity between the antibody and a peptide: all antigen-containing peptides are displayed as red circles, all other peptides as blue circles. The affinity is calculated as the area under the curve when antibody binding values are plotted against the corresponding peptide concentration. Each circle area is normalized to the peptide with the strongest affinity. Peptide Array, ab32356 binds to Histone H3 (di methyl K4) on Peptide Array but shows some weak non-specific binding to other Histone H3 modifications. We recommend ab176878 for Peptide Array of Histone H3 (di methyl K4).
The complete dataset, including full list of all peptides and information on the position of each peptide in the diagram, is available under the Product Protocol section.

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