abcam

ab203951

# Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free

Recombinant | RabMAb | Advanced Validation

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Constituents: PBS |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Carrier free** | Yes |
| **Clone number** | Y28 |
| **Purification technique** | Affinity purification Protein A |
| **Specificity** | This antibody has weak cross-reactivity with H3R8me1 and H3K4me3 in peptide array. Further optimization may be needed to minimize the cross-reactivity in assays similar to peptide array |
| **Concentration** | 1.044 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

## CUT&Tag

**Tested**

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | - |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Human, Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## PepArr

### Tested

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

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## IHC-P

### Tested

| | |
|---|---|
| **Species** | Human, Mouse, Rat |
| **Dilution info** | - |
| **Notes** | - |

## IP

### Expected

| | |
|---|---|
| **Species** | Mouse, Human |

| | |
|---|---|
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | - |
| **Notes** | - |

## ChIP

### Tested

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

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Mouse, Human, Rat |
| **Dilution info** | - |
| **Notes** | - |

## ICC/IF

### Tested

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

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

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## ChIP-seq

### Tested

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

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## Flow Cyt (Intra)

### Tested

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

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## ChIC/CUT&RUN-seq

### Tested

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

### Expected

| | |
|---|---|
| **Species** | Mouse, Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## Target data

See full target information H3C1 acetyl K9

**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 conditions** | +4°C |
| **Appropriate long-term storage conditions** | +4°C |
| **Storage information** | Do Not Freeze |

## Notes

ab203951 is the carrier-free version of ab32129.

**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.

**Conjugation ready**
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

**Compatibility**
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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## 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.

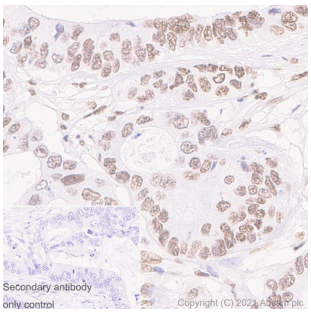
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We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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

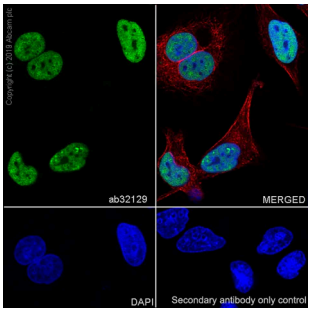
Full details and terms and conditions can be found here:
Terms & Conditions.

## 17 product images




### Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32129). Immunohistochemical analysis of Paraffin-embedded sections human cerebrum tissue labelling Histone H3 (acetyl K9) with ab32129 at 1/2000 dilution, followed by a ready to use secondary Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Staining on human cerebrum tissue is observed. Counter stained with Haematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

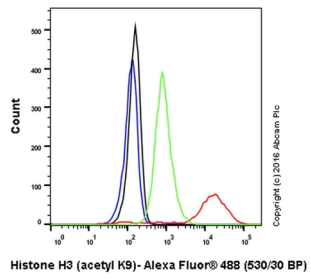



### Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

Histone H3 (acetyl K9) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Histone H3 (acetyl K9) antibody

Immunocytochemistry/ Immunofluorescence analysis of HeLa (human cervix adenocarcinoma epithelial cell) cells labeling Histone H3 with purified ab32129 at 1/250 dilution (4 µg/mL). Cells were fixed in 100% Methano. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain. ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/mL) was used as the secondary antibody only control.

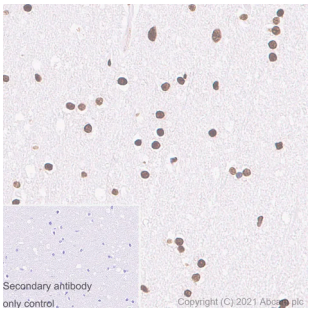
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32129).




### Flow Cytometry (Intracellular) - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

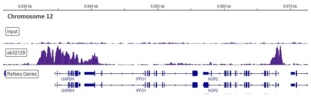
Histone H3 (acetyl K9) Flow Cytometry (Intracellular) staining using rabbit Anti-Histone H3 (acetyl K9) antibody

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) treated (Red)/untreated (Green) with 500ng/ml Trichostatin A for 4 hours with purified ab32129 at 1/230 dilution. The secondary antibody was Goat anti rabbit IgG (Alexa Fluorr® 488) at 1/2000 dilution. A Rabbit monoclonal IgG (Black) was used as the isotype control and cells without incubation with primary antibody and secondary antibody (Blue) were used as unlabeled control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32129).



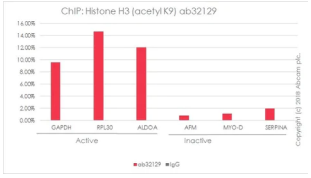

## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32129). Immunohistochemical analysis of Paraffin-embedded sections human colorectal carcinoma tissue labelling Histone H3 (acetyl K9) with ab32129 at 1/2000 dilution, followed by a ready to use secondary Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Staining on human colorectal carcinoma tissue is observed. Counter stained with Haematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



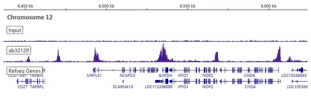

## ChIP-sequencing - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

This data was developed using the same antibody clone in a different buffer formulation (ab32129). 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 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129). 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.



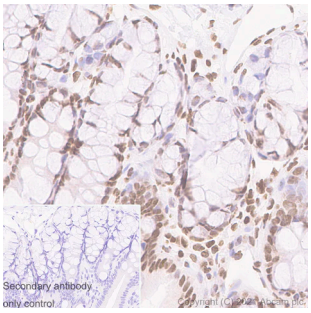

## ChIP - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

Chromatin was prepared from HeLa 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 ab32129 (red), and 20 µl of Protein A/G sepharose beads. No antibody was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach).
Primers and probes are located in the first kb of the transcribed region.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32129).



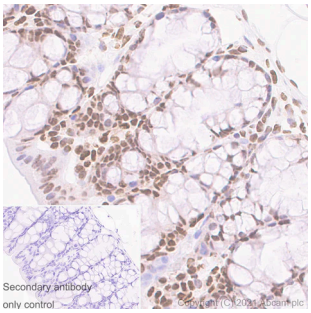

## ChIP-sequencing - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

This data was developed using the same antibody clone in a different buffer formulation (ab32129). Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 107 HeLa cells and 4 µg of Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129). 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.



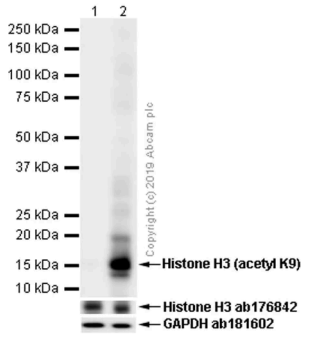

## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32129). Immunohistochemical analysis of Paraffin-embedded sections rat colon tissue labelling Histone H3 (acetyl K9) with ab32129 at 1/6000 dilution, followed by a ready to use secondary Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Staining on rat colon tissue is observed. Counter stained with Haematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.




## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32129). Immunohistochemical analysis of Paraffin-embedded sections mouse colon tissue labelling Histone H3 (acetyl K9) with ab32129 at 1/6000 dilution, followed by a ready to use secondary Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Staining on mouse colon tissue is observed. Counter stained with Haematoxylin. Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.




## Western blot - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32129). Blocking and diluting buffer and concentration: 5% NFDM/TBST. ab181602 was used as GAPDH loading control. ab176842 was for Histone H3 detection.
All lanes:
Western blot - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129) at 1/10000 dilution

Lane 1:
HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 15 µg

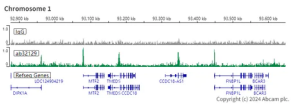
Lane 2:
HeLa (Human cervix adenocarcinoma epithelial cell) treated with 500ng/ml Trichostatin A for 4 hours whole cell lysate at 15 µg

Secondary

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

Predicted band size: 15 kDa

Observed band size: 15 kDa

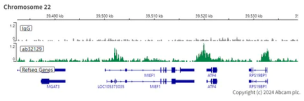


## ChIC/CUT&RUN sequencing - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human epithelial cell line from cervix adenocarcinoma) cells and 5 µg of ab32129 [Y28]. 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.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

This data was developed using the same antibody clone in a different buffer formulation (ab32129).

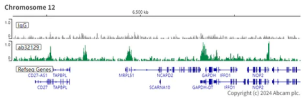


## ChIC/CUT&RUN sequencing - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human epithelial cell line from cervix adenocarcinoma) cells and 5 µg of ab32129 [Y28]. 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.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

This data was developed using the same antibody clone in a different buffer formulation (ab32129).

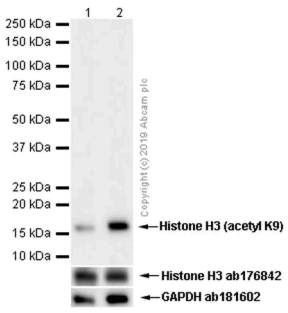


## ChIC/CUT&RUN sequencing - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human epithelial cell line from cervix adenocarcinoma) cells and 5 µg of ab32129 [Y28]. 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.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

This data was developed using the same antibody clone in a different buffer formulation (ab32129).




## Western blot - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32129). Blocking and diluting buffer and concentration: 5% NFDM/TBST. ab181602 was used as GAPDH loading control. ab176842 was for Histone H3 detection.
All lanes:
Western blot - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129) at 1/10000 dilution

Lane 1:

C6 (Rat glial tumor glial cell) whole cell lysate at 15 µg

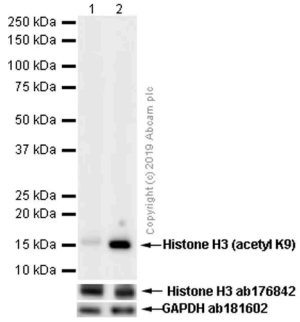
Lane 2:
C6 (Rat glial tumor glial cell) treated with 500ng/ml Trichostatin A for 4 hours whole cell lysate at 15 µg

Secondary

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

Predicted band size: 15 kDa

Observed band size: 15 kDa




## Western blot - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32129). Blocking and diluting buffer and concentration: 5% NFDM/TBST. ab181602 was used as GAPDH loading control. ab176842 was for Histone H3 detection.
All lanes:
Western blot - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129) at 1/10000 dilution

Lane 1:
NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate at 15 µg

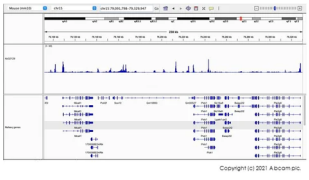
Lane 2:
NIH/3T3 (Mouse embryonic fibroblast) treated with 500ng/ml Trichostatin A for 4 hours whole cell lysate at 15 µg

Secondary

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

Predicted band size: 15 kDa

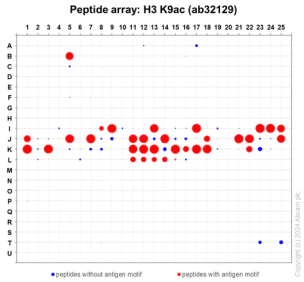
Observed band size: 15 kDa



This experiment and image is courtesy of Dr Marek Bartosovic, Gonçalo Castelo-Branco Group, Karolinska Institutet

## CUT&Tag - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

CUT&Tag-seq was performed using 200,000 Oli-neu (Oligodendrocyte progenitor) cells. Cells were permeabilized with 0.05% Digitonin and 0.01% NP-40 for 3 minutes. A 1:100 dilution of Recombinant Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade (ab32129) was used, along with a Guinea pig anti-rabbit Secondary. DNA was seg using Illumina NovaSeq S Prime to a depth of 24 million reads. This image is courtesy of Dr Marek Bartosovic, Gonçalo Castelo-Branco Group, Karolinska Institutet. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.




# Peptide Array - Anti-Histone H3 (acetyl K9) antibody [Y28] - ChIP Grade - BSA and Azide free (ab203951)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32129).

ab32129 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 area under curve when antibody binding values are plotted against the corresponding peptide concentration. Each circle area is normalized to the peptide with the strongest affinity.

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 this antibody has weak cross-reactivity with H3R8me1 and H3K4me3 in peptide array. Further optimization may be needed to minimize the cross-reactivity in assays similar to peptide array.

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