abcam

Product datasheet

Anti-Histone H2A.Z antibody - ChIP Grade ab4174

30 Abreviews 142 References 7 Images

Overview

Product name	Anti-Histone H2A.Z antibody - ChIP Grade
Description	Rabbit polyclonal to Histone H2A.Z - ChIP Grade
Host species	Rabbit
Specificity	From Jan 2024, QC testing of replenishment batches of this polyclonal changed. All tested and expected application and reactive species combinations are still covered by our Abcam product promise. However, we no longer test all applications. For more information on a specific batch, please contact our Scientific Support who will be happy to help.
Tested applications	**Suitable for:** ICC/IF, ChIP, WB
Species reactivity	**Reacts with:** Mouse, Rat, Cow, Human **Predicted to work with:** Sheep, Xenopus laevis, Arabidopsis thaliana, Zebrafish
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab11681**)
Positive control	ICC/IF: HepG2
General notes	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 Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab4174 in the following tested applications.

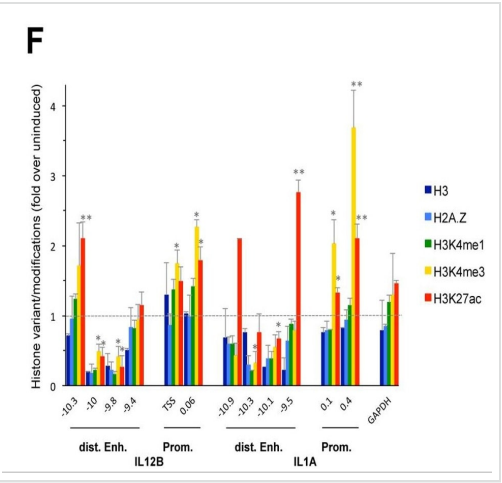
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★☆☆ (3)	1/1000.
ChIP	★★★★☆ (9)	Use at an assay dependent concentration.
WB	★★★★☆ (14)	1/1000. Detects a band of approximately 14 kDa (predicted molecular weight: 13.4 kDa). Abcam recommends using BSA as the blocking agent.

Target

Function	Variant histone H2A which replaces conventional H2A in a subset of nucleosomes. 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. May be involved in the formation of constitutive heterochromatin. May be required for chromosome segregation during cell division.
Sequence similarities	Belongs to the histone H2A family.
Post-translational modifications	Monoubiquitination of Lys-122 gives a specific tag for epigenetic transcriptional repression. Acetylated on Lys-5, Lys-8 and Lys-12 during interphase. Acetylation disappears at mitosis. Monomethylated on Lys-5 and Lys-8 by SETD6. SETD6 predominantly methylates Lys-8, lys-5 being a possible secondary site. Not phosphorylated.
Cellular localization	Nucleus. Chromosome.

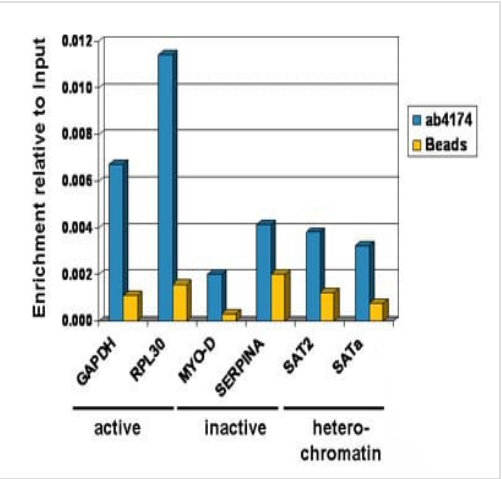
Images



ChIP - Anti-Histone H2A.Z antibody - ChIP Grade (ab4174)

Changes in nucleosome occupancy upon LPS induction at a putative distal enhancer and promoter of IL1A, kinetics of nucleosome removal, and changes in histone modifications.

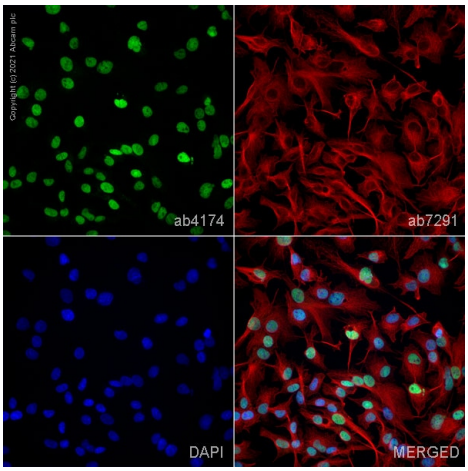
Chromation from mouse bone marrow derived macrophages. ChIP experiments with antibodies against H3 (dark blue bars), H2A.Z (light blue, (ab4174 at 4µg), H3K4me1 (green, **ab8895** at 1 µg), H3K4me3 (yellow, **ab8580** at 1 µg) and H3K27ac (red, **ab4729** at 1 µg). For these experiments cross-linked chromatin was lightly digested with MNase before incubation with the respective antibodies to increase resolution of the ChIP signal and the data was normalized to a region in the ORF of RPL4. Changes upon LPS induction in histone binding and histone modifications at the enhancers and promoters of IL12B and IL1A as well as at a control region in the GAPDH pseudo gene are shown as fold over levels found before induction. For H3K27ac the changes 1.5 h after LPS induction, and for all other histone variants and modifications the changes after 3 h of induction are shown. The error bars show the SEM of at least 3 independent experiments. Statistical significance of the changes in H3K4me3 and H3K27ac upon LPS induction compared to levels found prior to induction determined by *Student's* T-tests is indicated (*P<0.05; **P<0.01).



ChIP - Anti-Histone H2A.Z antibody - ChIP Grade (ab4174)

Chromatin was prepared from HeLa (Human epithelial cell line from cervix adenocarcinoma) cells according to the Abcam X-ChIP protocol.

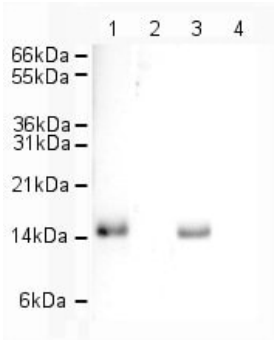
Cells were fixed with formaldehyde for 10 min. The ChIP was performed with 25 µg of chromatin, 2 µg of ab4174 (blue), and 20 µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are located in the first kb of the transcribed region.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A.Z antibody - ChIP Grade (ab4174)

ab4174 staining Histone H2A.Z in HepG2 cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-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 ab4174 at 1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). 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.



Western blot - Anti-Histone H2A.Z antibody - ChIP Grade (ab4174)

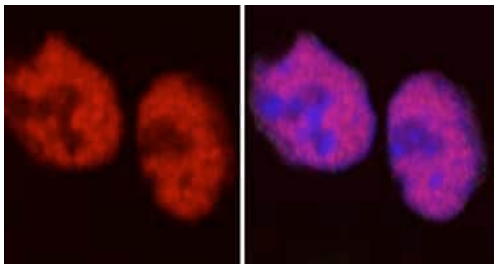
Lanes 1-2 : Anti-Histone H2A.Z antibody - ChIP Grade (ab4174) at 1/500 dilution
Lanes 3-4 : Anti-Histone H2A.Z antibody - ChIP Grade (ab4174) at 1/1000 dilution

Lanes 1 & 3 : Calf thymus histone lysate
Lanes 2 & 4 : Calf thymus histone lysate with Human Histone H2A.Z peptide (**ab11681**) at 1 µg/ml

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab6721**) at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 13.4 kDa



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A.Z antibody - ChIP Grade (ab4174)

Staining of Histone H2A.Z in mouse embryonic cells. The fixation is 2% PFA, and permeabilization is PBS 0.5% triton BSA. The dilution used was 1 in 100 (but it could be used at 1/200 to 1/300).

Red = H2A.Z

Blue = toto3 for the DNA

Western blot - Anti-Histone H2A.Z antibody - ChIP Grade (ab4174)

All lanes : Anti-Histone H2A.Z antibody - ChIP Grade (ab4174) at 1 µg/ml

Lane 1 : Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg
Lane 2 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate at 10 µg
Lane 3 : NIH/3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate at 10 µg
Lane 4 : PC-12 (Rat adrenal pheochromocytoma cell line) Whole Cell Lysate at 10 µg

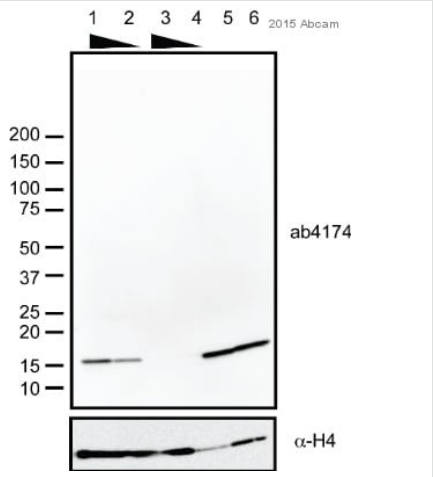
Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) (**ab65484**) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 13.4 kDa

Exposure time: 3 minutes



Western blot - Anti-Histone H2A.Z antibody - ChIP Grade (ab4174)

This image is courtesy of an Abreview submitted by Ragnhild Eskeland

All lanes : Anti-Histone H2A.Z antibody - ChIP Grade (ab4174) at 1/1000 dilution

Lane 1 : Native recombinant octamers K562 (Human chronic myelogenous leukemia cell line from bone marrow) cells at 3 µg
Lane 2 : Native recombinant octamers K562 cells at 1.5 µg
Lane 3 : Recombinant Human octamers containing H2A at 1 µg
Lane 4 : Recombinant Human octamers containing H2A at 0.5 µg
Lane 5 : Recombinant Human octamers containing H2A.Z.2.1 at 0.5 µg
Lane 6 : Recombinant Human octamers containing H2A.Z.1 at 0.5 µg

Secondary
All lanes : HRP-conjugated donkey anti-rabbit IgG polyclonal at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 13.4 kDa

Exposure time: 5 minutes

Blocked with 3% BSA for 1 hour at 20°C.

Primary incubation in TBS tween + 3% BSA at 20°C for 1 hour.

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