# abcam

### Product datasheet

# Anti-Histone H2A.Z antibody - ChIP Grade ab97966

★★★★★ 2 Abreviews 5 Images

#### Overview

Product name Anti-Histone H2A.Z antibody - ChIP Grade

**Description** Rabbit polyclonal to Histone H2A.Z - ChIP Grade

Host species Rabbit

Tested applications Suitable for: ICC/IF, WB, IHC-P, ChIP, IP

Species reactivity Reacts with: Human

Predicted to work with: Schizosaccharomyces pombe

**Immunogen** Synthetic peptide corresponding to Human Histone H2A.Z aa 65-128.

Positive control MOLT4 or Raji cell lysate. ICC/IF Hela cells IHC-P: Human normal colon FFPE tissue sections.

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. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw

cycles.

Storage buffer pH: 7.00

Preservative: 0.01% Thimerosal (merthiolate)

Constituents: 1.21% Tris, 0.75% Glycine, 10% Glycerol (glycerin, glycerine)

**Purity** Immunogen affinity purified

**Clonality** Polyclonal

**Isotype** IgG

# **Applications**

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#### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab97966 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		Use a concentration of 0.5 µg/ml.
WB		1/500 - 1/3000. Predicted molecular weight: 14 kDa.
IHC-P		Use a concentration of 0.1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ChIP	★ ☆ ☆ ☆ ☆ (1)	Use 2 µg for 25 µg of chromatin.
IP		1/500 - 1/1000.

#### **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

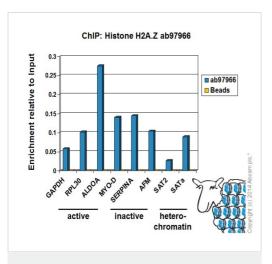
Monoubiquitination of Lys-122 gives a specific tag for epigenetic transcriptional repression. Acetylated on Lys-5, Lys-8 and Lys-12 during interphase. Acetylation disappears at mitosis. Monomethylated on Lys-5 and Lys-8 by SETD6. SETD6 predominantly methylates Lys-8, lys-5 being a possible secondary site.

Not phosphorylated.

#### **Cellular localization**

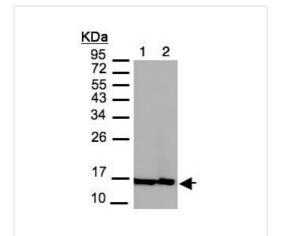
Nucleus. Chromosome.

# **Images**



ChIP - Anti-Histone H2A.Z antibody - ChIP Grade (ab97966)

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 25 $\mu$ g of chromatin, 2 $\mu$ g of ab97966 (blue), and 20 $\mu$ l 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.



Western blot - Anti-Histone H2A.Z antibody - ChIP Grade (ab97966)

**All lanes :** Anti-Histone H2A.Z antibody - ChIP Grade (ab97966) at 1/3000 dilution

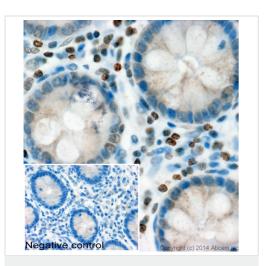
Lane 1 : MOLT4 whole cell lysate

Lane 2 : Raji whole cell lysate

Lysates/proteins at 30 µg per lane.

Predicted band size: 14 kDa





Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H2A.Z antibody - ChIP Grade (ab97966)

IHC image of ab97966 staining Histone H3 (phospho T45) in human colon formalin fixed paraffin embedded tissue sections\*, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab97966,  $1\mu g/ml$ , for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the negative control (shown on the inset).

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

\*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

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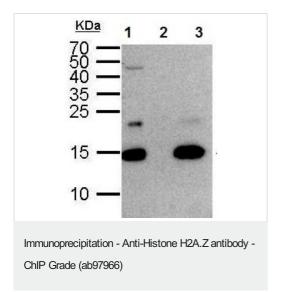
ab97966

DRAQS

-VE CONTROL

Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A.Z antibody - ChIP Grade (ab97966)

ab97966 staining Histone H2A.Z in HeLa cells. The cells were fixed with 100% methanol (5min) and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab97966 at 0.5μg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with an AlexaFluor®488 Goat anti-Rabbit secondary (ab150077) at 2 μg/ml (shown in green). AlexaFluor®350 WGA was used at a 1/200 dilution and incubated for 1h with the cells, to label plasma membranes (shown in blue). Nuclear DNA was labelled in red with 1.25 μM DRAQ5<sup>TM</sup> (ab108410), which was added to the secondary antibody mixture. A secondary only negative control is displayed, which indicates that the Histone H2A.Z staining observed is due to primary antibody specificity and not to unspecific binding of the secondary antibody to the cells.



ab97966 immunoprecipitating Histone H2A.Z protein in HeLa whole cell lysate/extract. Lane 1: 50  $\mu$ g HeLa whole cell lysate/extract. Lane 2: Control with 2  $\mu$ g of preimmune rabbit lgG. Lane 3: Immunoprecipitation of Histone H2A.Z protein by 2  $\mu$ g of ab97966. The immunoprecipitated Histone H2A.Z protein was detected with ab97966 diluted at 1:1000. Anti-rabbit lgG was used as a secondary antibody.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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- Replacement or refund for products not performing as stated on the datasheet
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- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise,

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