## Overview

<table>
<thead>
<tr>
<th><strong>Product name</strong></th>
<th>Anti-Histone H3 (tri methyl K4) antibody - ChIP Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description</strong></td>
<td>Rabbit polyclonal to Histone H3 (tri methyl K4) - ChIP Grade</td>
</tr>
<tr>
<td><strong>Host species</strong></td>
<td>Rabbit</td>
</tr>
<tr>
<td><strong>Tested applications</strong></td>
<td>Suitable for: ChIP/Chip, PepArr, ICC, ChIP, WB, IHC-Fr, IP, CHIPseq, Flow Cyt, ICC/IF, IHC-P</td>
</tr>
<tr>
<td><strong>Species reactivity</strong></td>
<td>Reacts with: Mouse, Rat, Rabbit, Human, Pig, Saccharomyces cerevisiae, Tetrahymena, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Zebrafish, Trypanosoma cruzi, Common marmoset, Rice, Xenopus tropicalis</td>
</tr>
<tr>
<td><strong>Immunogen</strong></td>
<td>Synthetic peptide within Human Histone H3 aa 1-100 (tri methyl K4) conjugated to Keyhole Limpet Haemocyanin (KLH). The exact sequence is proprietary. (Peptide available as ab92374)</td>
</tr>
<tr>
<td><strong>Positive control</strong></td>
<td>WB: Calf thymus histone preparation. ICC/IF: HeLa cells. IHC-P: Normal human colon tissue.</td>
</tr>
<tr>
<td><strong>General notes</strong></td>
<td>In immunofluorescence, a distinct property of tri methyl lysine 4 is its apparent 'ringing' of regions that appear as nucleoplasmic 'holes'. These represent the positions of splicing factor compartments, which often are easy to identify using only DNA stains in Indian muntjac fibroblasts. These splicing factor compartments are known to be preferentially associated with active genes and highly acetylated histone H3. The antibody, as expected, fails to stain heterochromatin (work by Kirk McManus, lab of Michael Hendzel). The immunofluorescence results suggest this antibody is an exceptional euchromatin probe.</td>
</tr>
</tbody>
</table>

## Properties

<table>
<thead>
<tr>
<th><strong>Form</strong></th>
<th>Liquid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Storage instructions</strong></td>
<td>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.</td>
</tr>
<tr>
<td><strong>Storage buffer</strong></td>
<td>pH: 7.40, Preservative: 0.02% Sodium azide, Constituent: PBS</td>
</tr>
</tbody>
</table>

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

Our **Abpromise guarantee** covers the use of ab8580 in the following tested applications.

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

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChIP/Chip</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>PepArr</td>
<td></td>
<td>Use a concentration of 0.2 - 0.02 µg/ml. Slight cross reactivity is observed with the Histone H3 - di methyl K4 modification. Optimisation is recommended to avoid array signal saturation.</td>
</tr>
<tr>
<td>ICC</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ChIP</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use 2 µg for 25 µg of chromatin.</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td></td>
<td>1/500.</td>
</tr>
<tr>
<td>IP</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>ChIPseq</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 20952408</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 19776550</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 1 µg/ml. 1/100 - 1/5000</td>
</tr>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. PubMed: 17634443</td>
</tr>
</tbody>
</table>

### Target

**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.

**Sequence similarities**
Belongs to the histone H3 family.

**Developmental stage**
Expressed during S phase, then expression strongly decreases as cell division slows down
during the process of differentiation.

**Post-translational modifications**

Acetylation is generally linked to gene activation. Acetylation on Lys-10 (H3K9ac) impairs methylation at Arg-9 (H3R8me2s). Acetylation on Lys-19 (H3K18ac) and Lys-24 (H3K24ac) favors methylation at Arg-18 (H3R17me).

Citrullination at Arg-9 (H3R8ci) and/or Arg-18 (H3R17ci) by PADI4 impairs methylation and represses transcription.

Asymmetric dimethylation at Arg-18 (H3R17me2a) by CARM1 is linked to gene activation. Symmetric dimethylation at Arg-9 (H3R8me2s) by PRMT5 is linked to gene repression. Asymmetric dimethylation at Arg-3 (H3R2me2a) by PRMT6 is linked to gene repression and is mutually exclusive with H3 Lys-5 methylation (H3K4me2 and H3K4me3). H3R2me2a is present at the 3’ of genes regardless of their transcription state and is enriched on inactive promoters, while it is absent on active promoters.

Methylation at Lys-5 (H3K4me3), Lys-37 (H3K36me) and Lys-80 (H3K79me) are linked to gene activation. Methylation at Lys-5 (H3K4me) facilitates subsequent acetylation of H3 and H4. Methylation at Lys-80 (H3K79me) is associated with DNA double-strand break (DSB) responses and is a specific target for TP53BP1. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are linked to gene repression. Methylation at Lys-5 (H3K4me) is a specific target for HP1 proteins (CBX1, CBX3 and CBX5) and prevents subsequent phosphorylation at Ser-11 (H3S10ph) and acetylation of H3 and H4. Methylation at Lys-5 (H3K4me) and Lys-80 (H3K79me) require preliminary monoubiquitination of H2B at ‘Lys-120’. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are enriched in inactive X chromosome chromatin.

Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and RPS6KA5 is important during interphase because it enables the transcription of genes following external stimulation, like mitogens, stress, growth factors or UV irradiation and result in the activation of genes, such as c-fos and c-jun. Phosphorylation at Ser-11 (H3S10ph), which is linked to gene activation, prevents methylation at Lys-10 (H3K9me) but facilitates acetylation of H3 and H4. Phosphorylation at Ser-11 (H3S10ph) by AURKB mediates the dissociation of HP1 proteins (CBX1, CBX3 and CBX5) from heterochromatin. Phosphorylation at Ser-11 (H3S10ph) is also an essential regulatory mechanism for neoplastic cell transformation. Phosphorylated at Ser-29 (H3S28ph) by MLTK isoform 1, RPS6KA5 or AURKB during mitosis or upon ultraviolet B irradiation. Phosphorylation at Thr-7 (H3T6ph) by PRKCBB is a specific tag for epigenetic transcriptional activation that prevents demethylation of Lys-5 (H3K4me) by LSD1/KDM1A. At centromeres, specifically phosphorylated at Thr-12 (H3T11ph) from prophase to early anaphase, by DAPK3 and PKN1. Phosphorylation at Thr-12 (H3T11ph) by PKN1 is a specific tag for epigenetic transcriptional activation that promotes demethylation of Lys-10 (H3K9me) by KDM4C/JMJD2C. Phosphorylation at Tyr-42 (H3Y41ph) by JAK2 promotes exclusion of CBX5 (HP1 alpha) from chromatin.

Monoubiquitinated by RAG1 in lymphoid cells, monoubiquitination is required for V(D)J recombination (By similarity). Ubiquitinated by the CUL4-DDB-RBX1 complex in response to ultraviolet irradiation. This may weaken the interaction between histones and DNA and facilitate DNA accessibility to repair proteins.

**Cellular localization**

Nucleus. Chromosome.

**Images**
Chromatin was prepared from U2OS 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 ab8580 (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.
All batches of ab8580 are tested in Peptide Array against peptides to different Histone H3 modifications. Six dilutions of each peptide are printed on to the Peptide Array in triplicate and results are averaged before being plotted on to a graph. Results show strong binding to Histone H3 - tri methyl K4 peptide (ab1342), indicating that this antibody specifically recognises the Histone H3 - tri methyl K4 modification. Slight cross reactivity is observed with the Histone H3 - di methyl K4 modification. Optimisation is recommended to avoid array signal saturation.

- ab1340 - Histone H3 - mono methyl K4
- ab1342 - Histone H3 - tri methyl K4
- ab1771 - Histone H3 - mono methyl K9
- ab1772 - Histone H3 - di methyl K9
- ab1773 - Histone H3 - tri methyl K9
- ab1780 - Histone H3 - mono methyl K27
- ab1781 - Histone H3 - di methyl K27
- ab1782 - Histone H3 - tri methyl K27
- ab7228 - Histone H3 - unmodified
- ab7768 - Histone H3 - di methyl K4
ab8580 staining Histone H3 (tri methyl K4) in HeLa cells. All 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 ab4729 at 1/1000 and ab7291 at 1µg/ml overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat anti-rabbit AlexaFluor®488 secondary (ab150077) at 2 µg/ml (shown in green) and a goat anti-mouse AlexaFluor®594 secondary (ab150120) at 2 µg/ml (shown in pseudo color red). Nuclear DNA was labelled in blue with DAPI.

Negative controls: 1– Rabbit primary and anti-mouse secondary antibody; 2 – Mouse primary antibody and anti-rabbit secondary antibody. Controls 1 and 2 indicate that there is no unspecific reaction between primary and secondary antibodies used.
IHC image of ab8580 staining Histone H3 (tri methyl K4) in normal 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 ab8580, 1/500 dilution, 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

Anti-Histone H3 (tri methyl K4) antibody - ChIP Grade (ab8580) at 1 µg/ml + Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg

Secondary
Goat Anti-Rabbit IgG (H+L) HRP-conjugated antibody at 1/50000 dilution

Performed under reducing conditions.

Predicted band size: 15 kDa
Observed band size: 17 kDa

Exposure time: 8 minutes
Western blot - Anti-Histone H3 (tri methyl K4) antibody - ChIP Grade (ab8580)

This image is courtesy of John E. Mueller and J. Ruth German (Mary Bryk lab)

All lanes: Anti-Histone H3 (tri methyl K4) antibody - ChIP Grade (ab8580) at 1/5000 dilution

Lane 1: S. cerevisiae whole cell lysate with WT
Lane 2: S. cerevisiae whole cell lysate with set1
Lane 3: S. cerevisiae whole cell lysate with bre2
Lane 4: S. cerevisiae whole cell lysate with sdc1
Lane 5: S. cerevisiae whole cell lysate with shg1
Lane 6: S. cerevisiae whole cell lysate with spp1
Lane 7: S. cerevisiae whole cell lysate with swd1
Lane 8: S. cerevisiae whole cell lysate with swd3

Lysates/proteins at 40 µg per lane.

Performed under reducing conditions.

Predicted band size: 15 kDa
Observed band size: 15 kDa

Rabbit polyclonal to Histone H3 tri methyl K9 (ab8580) at 1/5000 on S. cerevisiae whole cell lysate (40 ug per lane).

Protein resolved on 15% SDS-PAGE gel.
After transfer to PVDF membrane, blots were blocked in 1X PBS, 0.1% Tween-20, and 5% milk. ab1791 was diluted in 5 ml blocking buffer at 1/5000. Blots plus primary antibodies were either incubated overnight at 4C or at RT for 2 hr. Blots were washed 6X for 10 min each in PBS with 0.1% Tween-20 before addition of secondary antibodies. Secondary antibodies were diluted 1/2,000 in blocking buffer and incubated with blots for 2
hr at RT. Secondary blots were washed 4X for 10 min each in PBS with 0.1% Tween-20 and 2X for 10 min each in PBS.

Protein resolved on 15% SDS-PAGE gel. After transfer to PVDF membrane, blots were blocked in 1X PBS, 0.1% Tween-20, and 5% milk. ab1791 was diluted in 5 ml blocking buffer at 1/5000. Blots plus primary antibodies were either incubated overnight at 4°C or at RT for 2 hr.

ab8580 staining cultured human primary fibroblasts by ICC. Cells were PFA fixed and permeabilized in TritonX100 and saponin prior to blocking with 1% BSA for 1 hour at RT. The primary antibody was diluted 1/100 and incubated with the sample for 16 hours at 4°C. A FITC-conjugated rabbit anti-rabbit IgG antibody was used as the secondary.

Staining (green) with the anti-trimethyl Lysine K4 of Histone H3 antibody (ab8580) shows ringing of regions that appear as nucleoplasmic holes. These represent the positions of splicing factor compartments, which are preferentially associated with active genes and highly acetylated histone H3.

The antibody, as expected, fails to stain heterochromatin (red).
Mouse zygotes stained with the Tri-Methyl K4 histone H3 antibody (green) and DNA (blue). This modification is readily detected in the two pronuclei of the zygote.

*This image was kindly supplied as part of the review submitted by Dr Maria Elena Torres Padilla (University of Cambridge, UK).*

Human female lymphoblast immunostained with ab8580 (1:100) (yellowish green) specific for histone H3 lysine 4 (H3-K4) trimethylation; the DNA is stained red with propidium iodide (PI). Note the inactive X chromosome (arrow) and pericentromeric heterochromatin are largely devoid of this modification.

*This image is courtesy of Ahmad Khalil and Daniel Driscoll, University of Florida College of Medicine.*

**Please note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

**Our Abpromise to you: Quality guaranteed and expert technical support**

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- 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, please visit [http://www.abcam.com/abpromise](http://www.abcam.com/abpromise) or contact our technical team.

**Terms and conditions**
• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors