

Histone H3K4me2 antibody (pAb)

Catalog Nos: 39141, 39042, 39142

RRID: AB_2614985

Isotype: Serum

Application(s): ChIP, ChIP-Seq, CUT&Tag, DB, WB

Reactivity: Human, Mouse, Wide Range Predicted

Volumes: 100 µl, 50 µl, 10 µl

Purification: None

Host: Rabbit

Molecular Weight: 17 kDa

Background: Histone H3 is one of the core components of the nucleosome. The nucleosome is the smallest subunit of chromatin and consists of 147 base pairs of DNA wrapped around an octamer of core histone proteins (two each of Histone H2A, Histone H2B, Histone H3 and Histone H4). Histone H1 is a linker histone, present at the interface between the nucleosome core and DNA entry/exit points. Histone H1 is responsible for establishing higher-order chromatin structure. Chromatin is subject to a variety of chemical modifications, including post-translational modifications of the histone proteins and the methylation of cytosine residues in the DNA. Reported histone modifications include acetylation, methylation, phosphorylation, ubiquitylation, glycosylation, ADP-ribosylation, carbonylation and SUMOylation; these modifications play a major role in regulating gene expression. The methylation of histones can occur on two different residues: arginine or lysine. Histone methylation can be associated with transcriptional activation or repression, depending on the methylated residue. Lysine 4 of histone H3 can be mono-, di- or trimethylated by different histone methyltransferases (HMTs) such as SET1 or ASH1. Methylation of Lys4 is often associated with transcriptional activation. The demethylase LSD1 is able to demethylate histone H3 Lys4.

Immunogen: This Histone H3 dimethyl Lys4 antibody was raised against a peptide including dimethyl-lysine 4 of histone H3.

Buffer: Rabbit serum containing 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic. For your convenience, an IgG version (Catalog No. 39913) of this antibody that was purified by Protein A Chromatography is also available.

Application Notes:

Applications Validated by Active Motif:

ChIP: 5 - 10 µl per ChIP

ChIP-Seq: 3 µl each

WB*: 1:1,000 - 1:5,000 dilution

CUT&Tag: 1 µl per 50 µl reaction

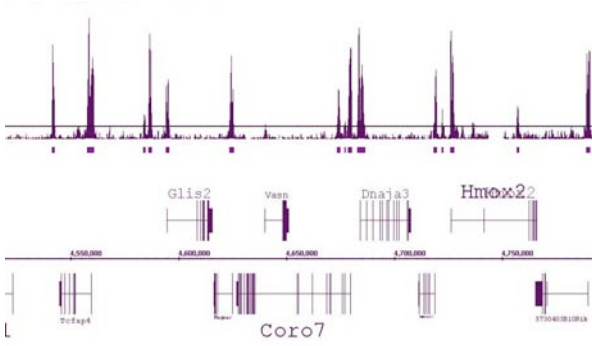
*Note: many chromatin-bound proteins are not soluble in a low salt nuclear extract and fractionate to the pellet. Therefore, we recommend a High Salt / Sonication Protocol when preparing nuclear extracts for Western Blot.

For Histone H3K4me2, we also offer AbFlex® Histone H3K4me2 Recombinant Antibody (rAb). For details, see Catalog No. 91321.

References: Histone H3K4me2 antibody has been cited in one or more publications. To see an up-to-date listing of papers that describe its use, please go to www.activemotif.com/catalog/details/39141.

Storage and Guarantee: Some products may be shipped at room temperature. This will not affect their stability or performance. Avoid repeated freeze/thaw cycles by aliquoting items into single-use fractions for storage at -20°C for up to 2 years. Keep all reagents on ice when not in storage. This product is guaranteed for 12 months from date of receipt.

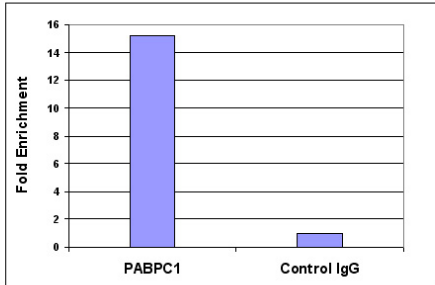
This product is for research use only and is not for use in diagnostic procedures.



Histone H3 dimethyl Lys4 antibody tested by ChIP-Seq.

ChIP was performed using the ChIP-IT[®] High Sensitivity Kit (Cat. No. 53040) and chromatin from 500,000 primary mouse T cells. ChIP DNA was sequenced on the Illumina GA II and 25 million sequence tags were mapped to identify H3K4me2 binding sites. The image shows the expected H3K4me2 binding pattern with enrichment at promoters and at locations outside of promoters.

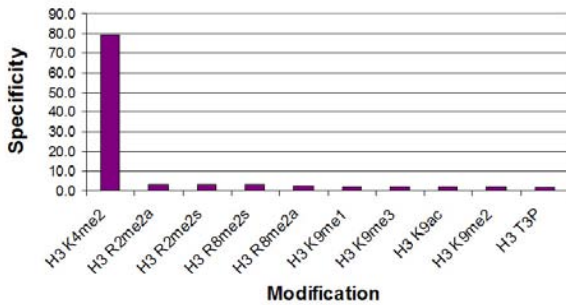
Histone H3 dimethyl Lys4 pAb tested by ChIP.



Chromatin IP performed using the ChIP-IT[®] Express Kit (Catalog No. 53008) and HeLa chromatin (1.5×10^6 cell equivalents per ChIP) using 15 μ l of Histone H3 dimethyl Lys4 antibody or the equivalent amount of rabbit IgG as a negative control. Real time, quantitative PCR (RT-qPCR) was performed on DNA purified from each of the ChIP reactions using a primer pair specific for the PABPC1 gene. Data are presented as Fold Enrichment of the ChIP antibody signal compared to the negative control IgG (which has been normalized to 1.0) using the ddCT method.

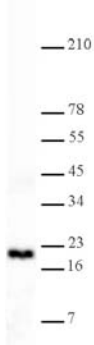
Histone H3 dimethyl Lys4 antibody specificity tested by peptide array analysis.

Peptide array analysis was used to confirm the specificity of this antibody for its intended modification. Histone H3 dimethyl Lys4 antibody was applied at a dilution of 1:10,000 to Active Motif's MODified[™] Histone Peptide Array (Catalog No. 13001). The arrays were scanned with ArrayAnalysis Software 7 and the results plotted. Specificity data is shown for the most reactive peptides and those related to the immunogen. Array Data File



Histone H3 dimethyl Lys4 antibody tested by Western blot.

HeLa acid extract (10 μ g) was probed with Histone H3 dimethyl Lys4 antibody (1:5,000 dilution).



Histone H3 dimethyl Lys4 pAb tested by dot blot analysis.

Dot blot analysis was used to confirm the specificity of Histone H3 dimethyl Lys4 pAb for dimethyl-lysine 4 of histone H3. Peptides corresponding to regions around major sites of histone H3 methylation (lysine 4, lysine 9, lysine 27) were spotted onto PVDF and probed with the antibody at a dilution of 1:5,000. The amount of peptide (in picomoles) spotted is indicated next to each row.



Lane 1: Unmodified Lys4 peptide. Lane 2: Monomethyl Lys4 peptide. Lane 3: Dimethyl Lys4 peptide. Lane 4: Trimethyl Lys4 peptide. Lane 5: Monomethyl Lys9 peptide. Lane 6: Unmodified Lys9 peptide. Lane 7: Dimethyl Lys9 peptide. Lane 8: Trimethyl Lys9 peptide. Lane 9: Unmodified Lys27 peptide. Lane 10: Monomethyl Lys27 peptide. Lane 11: Dimethyl Lys27 peptide. Lane 12: Trimethyl Lys27 peptide.