

## Histone H3K4me2 antibody (pAb)

**Catalog Nos:** 39913, 39914

**RRID:** AB\_2614976

**Application(s):** ChIP, DB, ICC, IF, WB

**Reactivity:** Human, Mouse, Wide Range Predicted

**Quantities:** 100 µg, 10 µg

**Purification:** Protein A Chromatography

**Host:** Rabbit

**Isotype:** IgG

**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:** Purified IgG in PBS (pH 7.5) with 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic. For your convenience, an unpurified serum version (Catalog No. 39141) of this antibody is also available.

### Application Notes:

Applications Validated by Active Motif:

ChIP: 10 µg per ChIP

ICC/IF: 1 - 2 µg/ml dilution

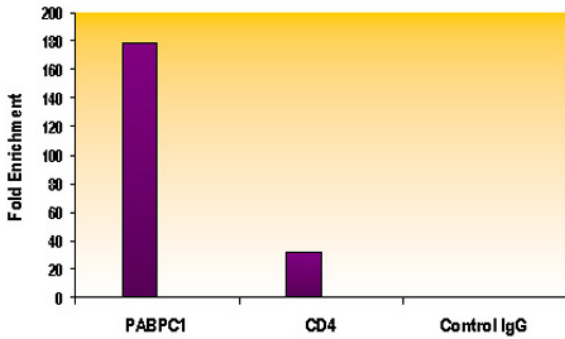
WB\*: 0.5 - 2 µg/ml dilution

DB: 1 µg/ml dilution

\*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.

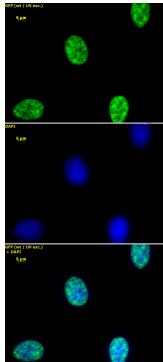
**Storage and Guarantee:** Some products may be shipped at room temperature. This will not affect their stability or performance. Avoid repeated freeze/thaw cycles by aliquoting items into single-use fractions for storage at -20°C for up to 2 years. Keep all reagents on ice when not in storage. This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



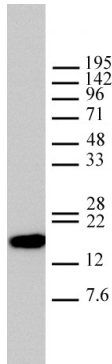
#### Histone H3K4me2 antibody (pAb) tested by ChIP analysis.

Chromatin IP performed using the ChIP-IT<sup>®</sup> Express Kit (Catalog No. 53008) and HeLa Chromatin (1.5 x 10<sup>6</sup> cell equivalents per ChIP) using 10 µg of Histone H3 dimethyl Lys4 pAb 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 indicated gene. Data are presented as Fold Enrichment of the ChIP antibody signal versus the negative control IgG using the ddCT method.



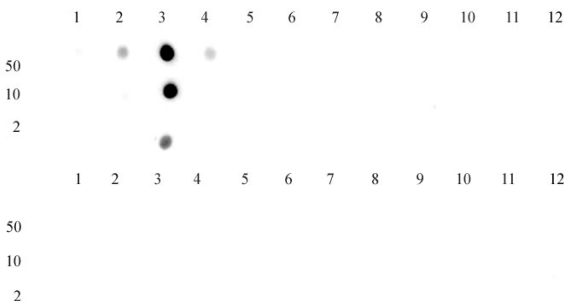
#### Histone H3K4me2 antibody (pAb) tested by immunofluorescence.

HeLa cells stained at 2 µg/ml with Histone H3 dimethyl Lys4 antibody. Top panel: Histone H3 dimethyl Lys4 antibody. Middle panel: DAPI. Bottom panel: merge.



#### Histone H3K4me2 antibody (pAb) tested by Western blot.

Nuclear extract of HeLa cells (20 µg) probed with Histone H3 dimethyl Lys4 antibody (1 µg/ml dilution).



#### Histone H3K4me2 antibody (pAb) tested by dot blot analysis.

Dot blot analysis was used to confirm the specificity of Histone H3 dimethyl Lys4 antibody for dimethyl-Lys4 of histone H3. Peptides corresponding to regions around major sites of histone H3 methylation were spotted onto PVDF and probed with Histone H3 dimethyl Lys4 antibody at a dilution of 1 µg/ml. The amount of peptide (in picomoles) spotted is indicated next to each row. Top panel: Lane 1: unmodified Lys4. Lane 2: monomethyl Lys4. Lane 3: dimethyl Lys4. Lane 4: trimethyl Lys4. Lane 5: unmodified Lys9, 14, 18. Lane 6: monomethyl Lys9. Lane 7: dimethyl Lys9. Lane 8: trimethyl Lys9. Lane 9: unmodified Lys79. Lane 10: monomethyl Lys79. Lane 11: dimethyl Lys79. Lane 12: trimethyl Lys79. Bottom panel: Lane 1: Unmodified Lys23. Lane 2: Monomethyl Lys23. Lane 3: Dimethyl Lys23. Lane 4: Trimethyl Lys23. Lane 5: unmodified Lys27. Lane 6: monomethyl Lys27. Lane 7: dimethyl Lys27. Lane 8: trimethyl Lys27. Lane 9: unmodified Lys36. Lane 10: monomethyl Lys36. Lane 11: dimethyl Lys36. Lane 12: trimethyl Lys36.