

Histone H3K9me2 antibody (pAb)

Catalog Nos: 39375, 39376

RRID: AB_2793234 **Isotype:** Serum

Application(s): ChIP, DB, ICC, IF, IP, WB **Reactivity:** Human, Wide Range Predicted

Volumes: 100 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 9 of histone H3 can be mono-, di- or trimethylated by different histone methyltransferases (HMTs) such as SuvH39H1 or G9a. This methylated lysine can be demethylated by histone demethylases as JMJD1A, LSD1 or JMJD2C. Methylation of this residue is mainly associated with transcriptional repression.

Immunogen: This Histone H3 dimethyl Lys9 antibody was raised against a peptide including dimethyl-lysine 9 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. 39753) of this antibody that was purified by Protein A Chromatography is also available.

Application Notes:

Applications Validated by Active Motif:

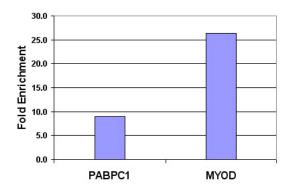
ChIP: 10 µl per ChIP

ICC/IF: 1:500 - 1:2,000 dilution WB: 1:5,000 - 1:10,000 dilution

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.

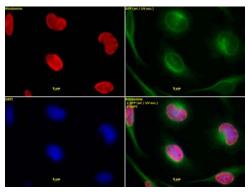
This product is for research use only and is not for use in diagnostic procedures.





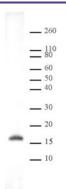
Histone H3 dimethyl Lys9 antibody tested by ChIP.

Chromatin IP performed using the ChIP-IT $^{\circ}$ Express Kit (Catalog No. 53008) and HeLa Chromatin (1.5 x 10 $^{\circ}$ cell equivalents per ChIP) using 10 μ I of Histone H3 dimethyl Lys9 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 either the PABPC1 gene or the MyoD gene. Data are presented as Fold Enrichment of the ChIP antibody signal versus the negative control IgG using the ddCT method.



Histone H3 dimethyl Lys9 antibody tested by immunofluorescence.

Top left: HeLa cells stained with Histone H3 dimethyl Lys9 antibody (1:1,000). Top right: Same cells stained with alpha Tubulin mAb (Clone 5-B-1-2). Bottom left: Same cells stained with DAPI. Bottom right: Merge of all 3 images.



Histone H3 dimethyl Lys9 antibody tested by Western blot.

HeLa acid extract (10 μ g per lane) probed with Histone H3 dimethyl Lys9 antibody at a 1:10,000 dilution.



Histone H3 dimethyl Lys9 antibody tested by dot blot analysis.

Dot blot analysis was used to confirm the specificity of Histone H3 dimethyl Lys9 antibody for dimethyl Lys9 histone H3. Methylated peptides corresponding to the immunogen and related sequences derived from histone H3 were spotted onto PVDF and probed with the antibody at 1:10,000. The amount of peptide (picomoles) spotted is indicated next to each row.

Top row:Lane 1: Unmod H3 aa 1-10. Lane 2: monomethyl Lys4. Lane 3: dimethyl Lys4. Lane 4: trimethyl Lys4. Lane 5: Unmod H3 aa 6-22. Lane 6: monomethyl Lys9. Lane 7: dimethyl Lys9. Lane 8: trimethyl Lys9. Bottom row: Lane 1: dimethyl Lys14. Lane 2: monomethyl Lys18. Lane 3: dimethyl Lys18. Lane 4: trimethyl Lys18. Lane 5: unmodified corresponding to amino acids 18 -27 of human histone H3. Lane 6: monomethyl Lys23. Lane 7: dimethyl Lys23. Lane 8: trimethyl Lys23. Lane 9: unmodified corresponding to amino acids 22-32 of human histone H3. Lane 10: monomethyl Lys27. Lane 11: dimethyl Lys27. Lane 12: trimethyl Lys27.