Histone H3K9me2 antibody (pAb)

Catalog Nos: 39239, 39041, 39240
RRID: AB_2793199
Isotype: Serum
Application(s): ChIP, DB, ICC, IF, 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 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.

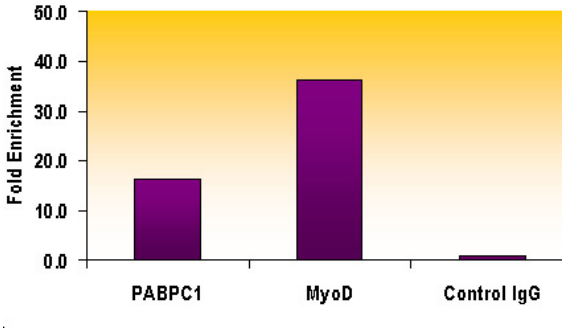
Buffer: Rabbit serum containing 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic.

Application Notes:
Applications Validated by Active Motif:
ChIP: 10 µl per ChIP
ICC/IF: 1:250 - 1:1,000 dilution
WB*: 1:2,000 - 1:5,000 dilution

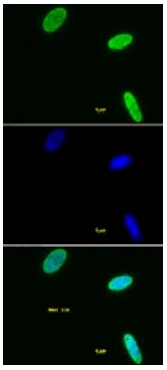
*Note: many chromatin-bound proteins are not soluble in a low salt nuclear extract and fractionate to the pellet. Therefore, we recommend a High Salt / Sonication Protocol when preparing nuclear extracts for Western blot.

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.

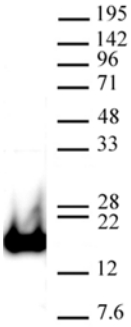
This product is for research use only and is not for use in diagnostic procedures.



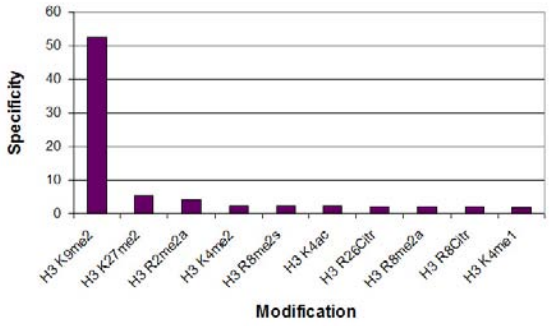
Histone H3 dimethyl Lys9 antibody tested by ChIP analysis.
Chromatin IP performed using the ChIP-IT® Express Kit (Catalog No. 53008) and HeLa Chromatin (1.5 x 10^6 cell equivalents per ChIP) using 10 µl of Histone H3 dimethyl Lys9 antibody or the equivalent amount of rabbit IgG as a negative control. Real time, quantitative PCR (RT-qPCR) was performed on DNA purified from each of the ChIP reactions using a primer pair specific for the indicated gene. Data are presented as Fold Enrichment of the ChIP antibody signal versus the negative control IgG using the ddCT method.



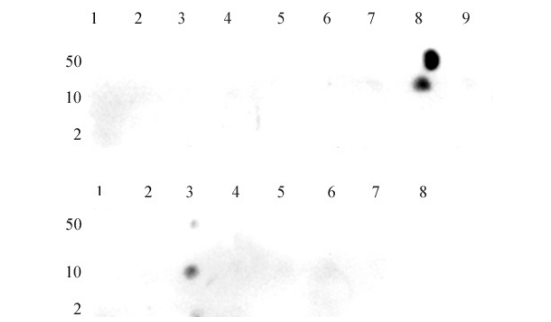
Histone H3K9me2 antibody tested by immunofluorescence.
Staining of HeLa cells with Histone H3K9me2 antibody (1:1,000 dilution, top panel) and DAPI (middle panel), and a merge of both images (bottom panel).



Histone H3K9me2 antibody tested by Western blot.
Detection of H3K9me2 by Western blot. The analysis was performed using 20 µg HeLa acid extract and Histone H3K9me2 antibody at a 1:1,000 dilution.



Histone H3K9me2 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 Lys9 antibody was applied at a dilution of 1:15,000 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.



Histone H3K9me2 antibody tested by dot blot analysis.
Decreasing amounts of peptides corresponding to regions around major sites of histone H3 methylation (lysine 4, lysine 9, lysine 27) were spotted onto PVDF and probed with antibody at 1:5,000.
Top Panel peptides - Lane 1: Unmod. K4. Lane 2: H3K4me1. Lane 3: H3K4me1,2. Lane 4: H3K4me2. Lane 5: H3K4me3. Lane 6: Unmod. K9. Lane 7: H3K9me1. Lane 8: H3K9me2. Lane 9: H3K9me3. Bottom Panel peptides – Lane 1: Unmod. H3. Lane 2: K27me1. Lane 3: K27me2. Lane 4: K27me3. Lane 5: Unmod.K36. Lane 6: K36me1. Lane 7: K36me2. Lane 8: K36me3.