

Histone H3K9me3 antibody (pAb)

Catalog Nos: 39161, 39062, 39162

RRID: AB_2532132

Isotype: Serum

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

Reactivity: Fission Yeast, Human, Wide Range Predicted

Volumes: 100 μ l, 50 μ l, 10 μ l

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 trimethyl Lys9 antibody was raised against a peptide including trimethyl-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. 39765) of this antibody that was purified by Protein A Chromatography is also available.

Application Notes:

Applications Validated by Active Motif:

ChIP: 2 - 10 μ l per ChIP

ChIP-Seq: 10 μ l each

ICC/IF: 1:500 - 1:1,000 dilution

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

CUT&Tag: 1 μ l per 50 μ l reaction

*This antibody has been validated for CUT&Tag using Active Motif's CUT&Tag-IT™ Assay Kit, Catalog No. 53160.

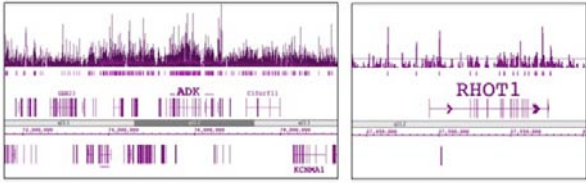
*Note: many chromatin-bound proteins are not soluble in a low salt nuclear extract and fractionate to the pellet. Therefore, we recommend a High Salt / Sonication Protocol when preparing nuclear extracts for Western blot.

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.

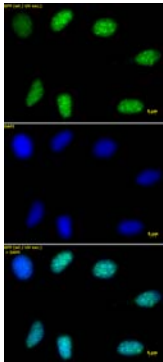
ChIP-Seq of Histone H3K9me3 pAb

ChIP was performed using the ChIP-IT[®] High Sensitivity Kit (Cat. No. 53040) with 15 µg of chromatin from D425 human Medulloblastoma cells and 10 µl of Histone H3K9me3 antibody (pAb). ChIP DNA was sequenced on the Illumina HiSeq and 16 million sequence tags were mapped to identify H3K9me3 binding sites. H3K9me3 binds broadly across the genome. The image shows two different regions. The left box shows many binding sites across a broad region on chromosome 1. The right box is zoomed in to show a dispersed binding pattern across the RHOT1 gene.



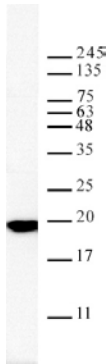
Histone H3 trimethyl Lys9 antibody tested by immunofluorescence.

HeLa cells stained at 2 µg/ml with Histone H3 trimethyl Lys9 antibody. Top panel: Histone H3 trimethyl Lys9 antibody. Middle panel: DAPI. Bottom panel: merge.



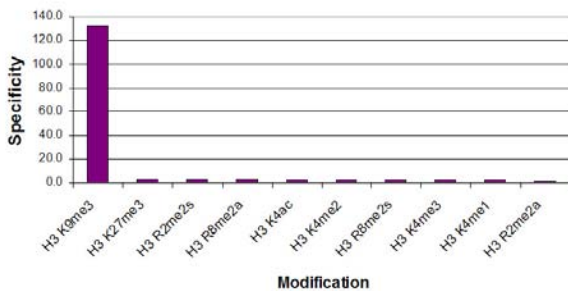
Histone H3K9me3 antibody (pAb) tested by Western blot.

HeLa cell nuclear extract (20 µg per lane) probed with Histone H3K9me3 antibody (pAb) at a dilution of 1:5,000.



Histone H3 trimethyl Lys9 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 trimethyl Lys9 antibody was applied at a dilution of 1:5,000 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.



Dot blot of Histone H3K9me3 antibody.

Peptides were spotted onto PVDF and probed with H3K9me3 at 1:5000. The amount of protein (picomoles) spotted is indicated next to each row.

Top panel - Col 1: H3K4me1 aa 1-10. Col 2: H3K4me2 aa 1-10. Col 3: H3K4me3 aa 1-10. Col 4: unmod K9 aa 4-14. Col 5: K9me1 aa 6-15. Col 6: K9me2 aa 6-15. Col 7: K9me3 aa 6-15. Col 8: K9me1 aa 4-13. Col 9: K9me2 aa 4-13. Col 10: K9me3 aa 4-13. Bottom panel - Col 1: unmod K27 aa22-32. Col 2: H3K27me1 aa 24-36. Col 3: H3K27me2 aa 24-36. Col 4: H3K27me3 aa 24-36. Col 5: unmod K36 aa 30-38. Col 6: H3K36me1 aa30-38. Col 7: H3K36me2 aa30-38. Col 8: H3K36me3 aa30-38.

