Histone H3K27me2me3 antibody (mAb)

Catalog Nos: 39535, 39435, 39537
RRID: AB_2793246
Clone: 7B11
Application(s): ChIP, ChIP-Seq, CUT&Tag, IF, WB
Reactivity: Human, Mouse, Wide Range Predicted

Volumes: 100 µl, 50 µl, 10 µl
Purification: Ascites
Host: Mouse
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; it 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; they play a major role in regulating gene expression.Histone H3K27 can be mono-, di- or trimethylated by different histone methyltransferases, such as EZH2 or NSD3. While histone methylation can be associated with transcriptional activation or repression, methylation of Lysine 27 of histone H3 is mainly associated with transcriptional repression.

Immunogen: This Histone H3 di/trimethyl Lys27 antibody was raised against a peptide including trimethyl-lysine 27 of histone H3.

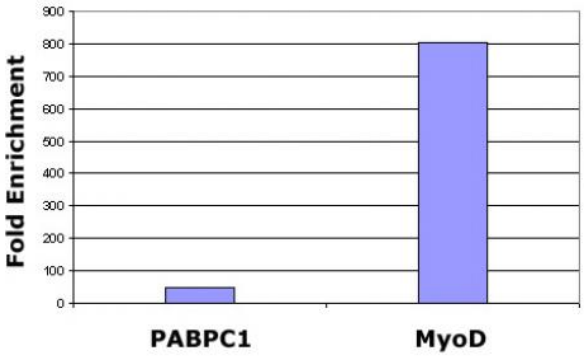
Buffer: Ascites containing 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic. For your convenience, an IgG version (Catalog No. 39536) of this antibody that was purified by Protein A Chromatography is also available.

Application Notes:
Applications Validated by Active Motif:
ChIP: 1 - 3 µl per ChIP
WB*: 1:500 - 1:2,000 dilution
CUT&Tag: 1-2 µl per 50 µl reaction

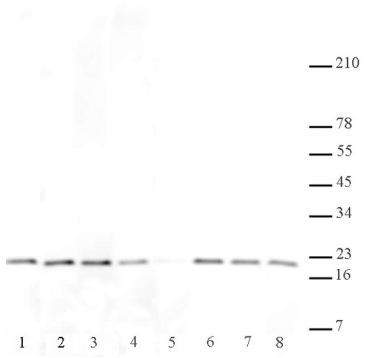
*Note: many chromatin-bound proteins are not soluble in a low salt nuclear extract and fractionate to the pellet. Therefore, we recommend a High Salt / Sonication Protocol when preparing nuclear extracts for Western Blot.

The modENCODE and NIH Roadmap Epigenomics Mapping Consortiums have implemented rigorous standardization criteria for all assays and reagents to be used. As part of this initiative, antibody specificity testing and the ability of the antibodies to work in ChIP-Seq were assessed in a large-scale study. This Histone H3 di/trimethyl Lys27 antibody was validated for ChIP-Seq in the study (see reference).

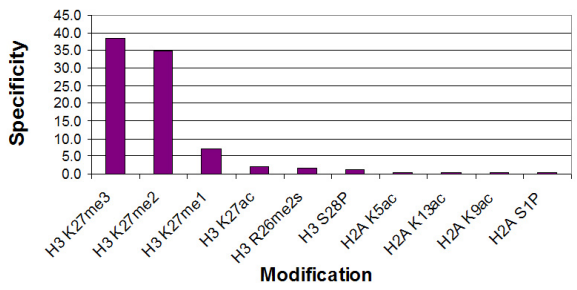
Storage and Guarantee: Some products may be shipped at room temperature. This will not affect their stability or performance. Avoid repeated freeze/thaw cycles by aliquoting items into single-use fractions for storage at -20°C for up to 2 years. Keep all reagents on ice when not in storage. This product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



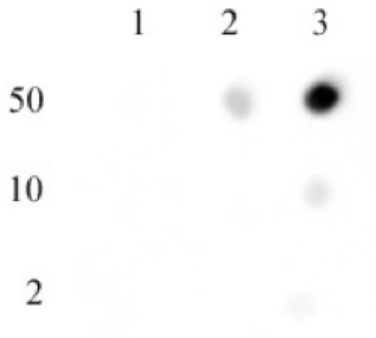
Histone H3 di/trimethyl Lys27 antibody (mAb) tested by ChIP analysis.
Chromatin IP performed using the ChIP-IT® Express Kit (Catalog No. 53008) and HeLa Chromatin (1.5 x 10^6 cell equivalents per ChIP) using 10 µl of Histone H3 di/trimethyl Lys27 antibody (mAb) or 2 µg of rabbit IgG as a negative control. 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.



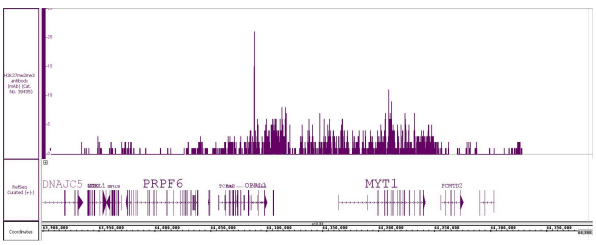
Histone H3 di/trimethyl Lys27 antibody (mAb) tested by Western blot.
HeLa acid extract (10 µg per lane) was probed with Histone H3 di/trimethyl Lys27 antibody (mAb) at a 1:2,000 dilution with or without pre-incubation of antibody with 1 µM peptide containing the sequence surrounding either lysine 9 or lysine 27 of Histone H3.
Lane 1: No peptide.
Lane 2: Unmodified Lys27 peptide.
Lane 3: Monomethyl Lys27 peptide.
Lane 4: Dimethyl Lys27 peptide.
Lane 5: Trimethyl Lys27 peptide.
Lane 6: Monomethyl Lys9 peptide.
Lane 7: Dimethyl Lys9 peptide.
Lane 8: Trimethyl Lys9 peptide.



Histone H3 di/trimethyl Lys27 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 di/trimethyl Lys27 antibody was applied at a dilution of 1:500 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 H3 di/trimethyl Lys27 antibody specificity tested by Dot blot.
Recombinant Histone samples were spotted onto positively charged nylon membrane and blotted with Histone H3 di/trimethyl Lys27 antibody at a dilution of 1:1000. Lane 1: Recombinant Histone H3K27me1 (MLA) Cat. No. 31214. Lane 2: Recombinant Histone H3K27me2 (MLA) Cat. No. 31215. Lane 3: Recombinant Histone H3K27me3 (MLA) Cat. No. 31216. The results show that the antibody detects di-methyl and tri-methyl Histone H3K27, but not the mono-methylated form.



Histone H3 di/trimethyl antibody (mAb) tested by CUT&Tag
CUT&Tag was performed using 100,000 K562 cells and sequenced using 38 base-pair, paired-end reads on the Illumina NovaSeq. Data was collected from 5 million reads, and H3K27me2me3 data is shown for Chromosome