Histone H3K4me3 antibody (pAb)

Catalog Nos: 39915, 39016, 39916
RRID: AB_2687512
Application(s): ChIP, ChIP-Seq, CUT&RUN, DB, ICC, IF, WB
Reactivity: Budding Yeast, Human, Wide Range Predicted

Quantities: 100 µg, 50 µg, 10 µg
Purification: Protein A Chromatography
Host: Rabbit
Isotype: IgG
Concentration: 1 µg/µl
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 trimethyl Lys4 antibody was raised against a peptide including trimethyl-lysine 4 of histone H3.

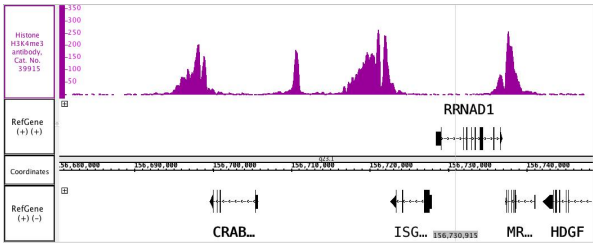
Buffer: Purified IgG in PBS (pH 7.5) with 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic. For your convenience, an unpurified serum version (Catalog No. 39159) of this antibody is also available.

Application Notes:
Applications Validated by Active Motif:
ChIP: 3 µg per ChIP
ChIP-Seq: 4 µg each
ICC/IF: 1 - 2 µg/ml dilution
WB*: 2 - 4 µg/ml dilution
CUT&Tag: 1 µl per 50 µl reaction*
CUT&RUN: 1 µl per 50 µl reaction

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

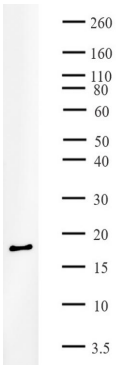
*Note: many chromatin-bound proteins are not soluble in a low salt nuclear extract and fractionate to the pellet. Therefore, we recommend a High Salt / Sonication Protocol when preparing nuclear extracts for Western Blot.

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.



Histone H3K4me3 antibody tested by ChIP-Seq

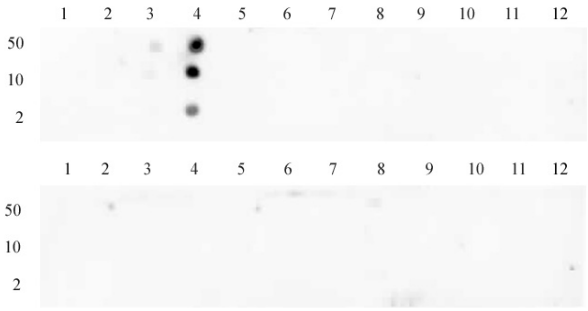
Chromatin immunoprecipitation (ChIP) was performed using the ChIP-IT® High Sensitivity Kit (Cat. No. 53040) with Acute Myelocytic Leukemia (AML) cell line chromatin and 4 µg of Histone H3K4me3 antibody. ChIP DNA was sequenced on the Illumina NextSeq and 14.5 million sequence tags were mapped to identify Histone H3K4me3 binding sites on chromosome 12.



Histone H3K4me3 antibody tested by Western blot.

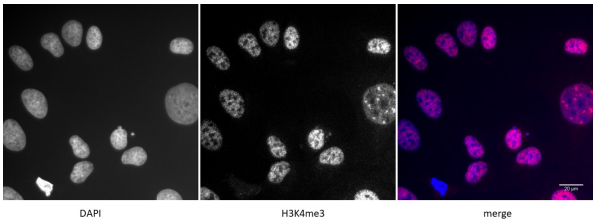
The analysis was performed using 20 µg HeLa nuclear cell extract and Histone H3K4me3 antibody at 2 µg/ml.

*For optimal results, we recommend a High Salt & Sonication Protocol when preparing nuclear extracts. Visit activemotif.com to download the protocol.



Histone H3 trimethyl Lys4 antibody tested by dot blot analysis.

Dot blot analysis was used to confirm the specificity of Histone H3 trimethyl Lys4 antibody for trimethyl-Lys4 of histone H3. Peptides corresponding to regions around major sites of histone H3 methylation were spotted onto PVDF and probed with Histone H3 trimethyl 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: dimethyl Lys14. Lane 10: monomethyl Lys18. Lane 11: dimethyl Lys18. Lane 12: trimethyl Lys18. 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.



Detection of H3K4me3 by immunofluorescence

U2OS cells were stained with H3K4me3 antibody at a dilution of 1:500. Left panel: DAPI. Middle panel: H3K4me3 antibody staining Right panel: merge.