## Histone H3K56ac antibody (pAb)



### Catalog Nos: 39281, 39082, 39282 RRID: AB\_2661786 Application(s): ChIP, ChIP-Seq, CUT&Tag, DB, WB Reactivity: Budding Yeast, Human, Wide Range Predicted

Volumes: 100 µl, 50 µl, 10 µl Purification: Affinity Purified Host: Rabbit 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.

Lysine N- $\epsilon$ -acetylation is a dynamic, reversible and tightly regulated protein and histone modification that plays a major role in chromatin remodeling and in the regulation of gene expression in various cellular functions. Acetylation of histone H3 occurs at several different lysine positions in the histone tail, and is performed by Histone Acetyltransferases (HATs) such as CBP/p300. Acetylation of histones is often associated with transcriptional activation. Histone H3 Lys56 acetylation occurs normally during S phase, but disappears in G<sub>2</sub>. This modification persists in presence of DNA damage and also plays a role in nucleosome assembly. Rtt109 was shown to be the major histone acetyltransferase (HAT) for Lys56 acetylation.

Immunogen: This Histone H3 acetyl Lys56 antibody was raised against peptide containing acetyl-Lys56 of yeast histone H3.

**Buffer:** Purified rabbit IgG in 70 mM Tris (pH 8), 105 mM NaCl, 31 mM glycine, 0.07 mM EDTA, 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic.

### **Application Notes:**

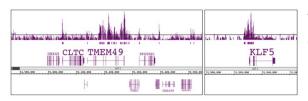
Applications Validated by Active Motif: ChIP: 3 - 5 µl per ChIP ChIP-Seq: 10 µl each WB: 1:2,500 - 1:5,000 dilution CUT&Tag: 1-2 µl per 50 µl reaction

For Histone H3K56ac, we also offer AbFlex<sup>®</sup> Histone H3K56ac Recombinant Antibody (rAb). For details, see Catalog No. 91127.

**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.

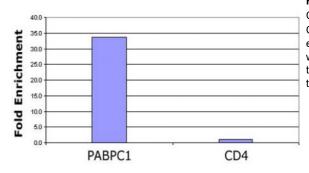
This product is for research use only and is not for use in diagnostic procedures.





#### Histone H3K56ac antibody (pAb) tested by ChIP-chip.

ChIP was performed using the ChIP-IT<sup>®</sup> High Sensitivity Kit (Cat. No. 53040) with chromatin from 3 million HeLa cells and 5 µl of antibody. ChIP DNA was amplified by WGA, labeled and hybridized to a human tiling array. The image on the left shows H3K56Ac binding across a 650,000 bp region on chromosome 17. The image on the right shows H3K56Ac binding at the KLF5 gene.



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#### Histone H3K56ac antibody (pAb) tested by ChIP analysis.

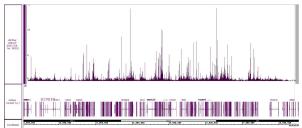
Chromatin IP performed using the ChIP-IT<sup>®</sup> Express Kit (Catalog No. 53008) and HeLa Chromatin (1.5 x 10<sup>6</sup> cell equivalents per ChIP) using 2  $\mu$ I of Histone H3 acetyl Lys56 pAb or the equivalent amount of rabbit IgG as a negative control. Real time, quantitative PCR (RT-qPCR) 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 H3K56ac antibody tested by Western blot.

HeLa acid extract (20 µg/lane) was probed with Histone H3 acetyl Lys56 polyclonal antibody (1:5,000 dilution).

Lane 1: No treatment. Lane 2: Cells treated with sodium butyrate.

#### Histone H3K56ac antibody (pAb) tested by CUT&Tag



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CUT&Tag was performed using 100,000 K562 cells and sequenced using 38 base-pair, pairedend reads on the Illumina NovaSeq. Data was collected from 7 million reads, and H3K56ac data is shown for Chromosome 1.

# 1 2 3 4 5 6 7 8 9 10 11 12 250 50 10 2

#### Histone H3K56ac antibody (pAb) tested by dot blot analysis.

Dot blot analysis was used to confirm the specificity of Histone H3 acetyl Lys56 pAb for acetyl Lys56 histone H3. Acetylated peptides corresponding to the immunogen and related peptides were spotted onto PVDF and probed with the antibody at 1:20,000. The amount of peptide (picomoles) spotted is indicated next to each row.

Lane 1: H3 acetyl-Lys4 peptide. Lane 2: H3 acetyl-Lys9 peptide. Lane 3: H3 acetyl-Lys14 peptide. Lane 4: H3 acetyl-Lys18 peptide. Lane 5: unmodified H3 peptide aa 13-22. Lane 6: H3 acetyl-Lys23 peptide. Lane 7: H3 acetyl-Lys27 peptide. Lane 8: H3 acetyl-Lys36 peptide. Lane 9: H3 acetyl-Lys37 peptide. Lane 10: yeast H3 acetyl-Lys56 peptide. Lane 11: unmodified yeast H3 peptide aa 50-59. Lane 12: human H3 acetyl-Lys56 peptide.