

AbFlex® Histone H3K27ac antibody (rAb)

Catalog Nos: 91193, 91194

RRID: AB_2793797

Application(s): ChIP-Seq, CUT&RUN, CUT&Tag, ELISA, WB

Reactivity: Human, Wide Range Predicted

Quantities: 100 µg, 10 µg

Purification: Protein A Chromatography

Host: Mouse Isotype: IgG2a

Concentration: 1 µg/µl Molecular Weight: 17 kDa

Background: AbFlex[®] antibodies are recombinant antibodies (rAbs) that have been generated using defined DNA sequences to produce highly specific, reproducible antibodies. Each AbFlex antibody contains a 6xHis Tag, a Biotinylation Tag for enzymatic biotin conjugation using the biotin ligase, BirA, and a sortase recognition motif (LPXTG) to attach a variety of labels directly to the antibody including fluorophores, enzymatic substrates (HRP, AP), peptides, drugs as well as solid supports.

AbFlex[®] Histone H3K27ac antibody was expressed as full-length IgG with mouse immunoglobulin heavy and light chains (IgG2a isotype) in mammalian 293 cells.

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). 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.

Immunogen: This antibody was raised against a synthetic peptide including acetyl-lysine 27 of human histone H3.

Buffer: Purified IgG in 140 mM Hepes, pH 7.5, 70 mM NaCl, 32 mM NaOAc, 0.035% sodium azide, 30% glycerol. Sodium azide is highly toxic.

Application Notes:

Applications Validated by Active Motif:

ChIP-Seq: 4 µg per ChIP

WB*: 0.2 - 2 μg/ml

Bead-based ELISA: 1 - 10 µg/ml CUT&Tag: 1 µl per 50 µl reaction CUT&RUN: 1 µl per 50 µl reaction

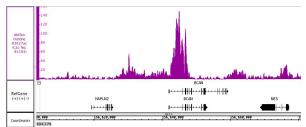
*Note: many chromatin-bound proteins are not soluble in a low salt nuclear extract and fractionate to the pellet. Therefore, we recommend a High Salt / Sonication Protocol when preparing nuclear extracts for Western Blot.

We offer a polyclonal version of Histone H3K27ac. For details, see Catalog No. 39685.

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.

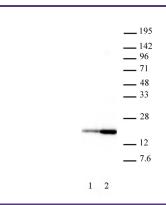
This product is for research use only and is not for use in diagnostic procedures.





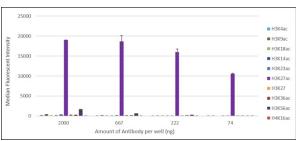
AbFlex® Histone H3K27ac antibody tested by ChIP-Seq.

Chromatin immunoprecipitation (ChIP) was performed using the <u>ChIP-IT[®] High Sensitivity</u> <u>Kit</u> (Cat. No. 53040) with 30 μg of chromatin from HAP1 cells and 4 μg Histone H3K27ac antibody. ChIP DNA was sequenced on the Illumina HiSeq and 17.8 million sequence tags were mapped to identify H3K27ac binding sites.



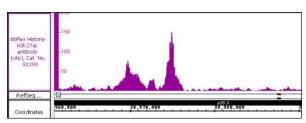
AbFlex® Histone H3K27ac antibody tested by Western Blot

HeLa cells were treated with 5 mM sodium butyrate for 19 hrs. (Lane 2) or untreated (Lane 1). Following treatment, 20 μ g of HeLa cell nuclear extract was loaded on SDS-PAGE gel and probed with 1 μ g/ml AbFlex Histone H3K27ac antibody. MW: 17 kDa



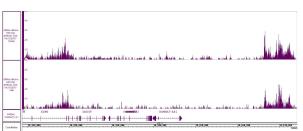
AbFlex Histone H3K27ac antibody, tested by Dot Blot

Dot blot analysis was used to confirm the specificity of AbFlex Histone H3K27ac antibody for acetylated H3K27. Recombinant Histone Proteins were spotted onto PVDF and probed with the antibody at 1 μg/ml. The amount of protein (picomoles) spotted is indicated next to each row. Columns as follows: 1) H3K37ac 2) Histone H3K36ac 3) Histone H3K9ac 4) Histone H3K14ac 5) Histone H3K18ac 6) Histone H3K23ac 7) Histone Unmod H3K27 8) Histone H3K27ac 9) Histone H4K5ac 10) Histone H4K8ac 11) Histone H4K12ac 12) Histone H4K16ac



AbFlex® Histone H3K27ac antibody tested by CUT&Tag

CUT&Tag was performed using 250,000 K562 cells and sequenced using 38 base-pair, pairedend reads on the Illumina NextSeq 500/550. Data was collected from 31 million reads, and Histone H3K27ac data is shown for Chromosome 1.



AbFlex® H3K27ac antibody (rAb) tested by CUT&RUN

CUT&RUN was performed using 500,000 K562 nuclei (TOP) or 500,000 K562 cells (BOTTOM) and sequenced using 38 base-pair, paired-end reads on the Illumina NovaSeq. Data was collected from 24 million reads (TOP) and 26 million reads (BOTTOM). H3K4me3 data is shown for Chromosome 19.