

## AbFlex® Histone H3K9me0 antibody (rAb)

**Catalog Nos:** 91155, 91156

**RRID:** AB\_2793790

**Application(s):** ChIP, ChIP-Seq, ELISA, ICC, IF, WB

**Reactivity:** Human, Wide Range Predicted

**Quantities:** 100 µg, 10 µg

**Purification:** Ni-NTA

**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 H3K9me0 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 basic building block of chromatin. Histones are 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.

Methylation of lysines occur as four states: unmethylated (me0), monomethyl (me1), dimethyl (me2) and trimethyl (me3). The me0 state of lysine is recognized as biologically relevant and a number of proteins containing PhD fingers, ADD and WD40 domains are known to associate with unmodified lysines.

**Immunogen:** This antibody was raised against a synthetic branched peptide corresponding to amino acids surrounding Lys9 of human Histone H3.

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

### Application Notes:

Applications Validated by Active Motif:

ICC/IF: 1 - 2 µg/ml

WB\*: 0.1 - 1 µg/ml

Bead-based ELISA: 0.5 - 10 µg/ml

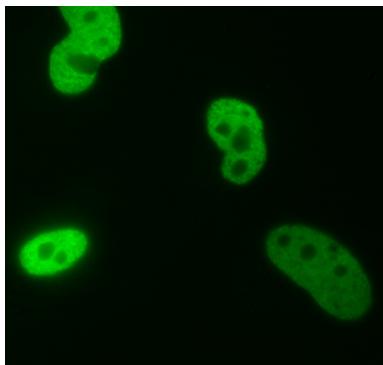
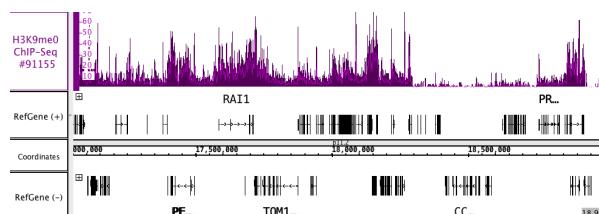
\*Note: many chromatin-bound proteins are not soluble in a low salt nuclear extract and fractionate to the pellet. Therefore, we recommend a High Salt / Sonication Protocol when preparing nuclear extracts for Western Blot.

**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.

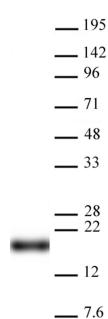
AbFlex® Histone H3K9me0 Antibody (rAb) tested by ChIP-Seq.

ChIP was performed using the ChIP-IT® High Sensitivity Kit (Cat. No. 53040) with 30 µg of chromatin from PC9, lung adenocarcinoma, cells and 4 µg of antibody. ChIP DNA was sequenced on the Illumina HiSeq and 30 million sequence tags were mapped to identify Histone H3K9me0 binding sites. The image shows binding across a region of chromosome 17.



AbFlex® Histone H3K9me0 Antibody (rAb) tested by immunofluorescence.

HeLa cell stained with 2µg /mL of AbFlex® Histone H3K9me0 Antibody (rAb followed by anti-mouse-IgG-488.



AbFlex® Histone H3K9me0 antibody (rAb) tested by Western blot.

HeLa nuclear extract (20 µg per lane) probed with AbFlexTM Histone H3K9me0 antibody (0.2 µg/ml dilution).

AbFlex® Histone H3K9me0 antibody (rAb) tested by Luminex bead-based specificity analysis. Luminex bead-based specificity analysis was used to confirm the specificity of AbFlex® Histone H3K9me0 antibody (rAb) antibody for Histone H3K9me0. Various Histone H3 recombinant proteins were conjugated to MagPlex Luminex beads and incubated with various amounts of AbFlex® Histone H3K9me0 antibody (rAb). Protein-bound antibody was detected with anti-mouse IgG-Phycoerythrin and read in a Luminex instrument.

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