

## AbFlex® Histone H3K4me2 antibody (rAb)

**Catalog Nos:** 91321, 91322

**RRID:** AB\_3216329

**Application(s):** ChIP, ChIP-Seq, WB

**Reactivity:** Human, Wide Range Predicted

**Quantities:** 100 µg, 10 µg

**Purification:** Protein A Chromatography

**Host:** Mouse

**Isotype:** IgG2a

**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® H3K4me2 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). 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 antibody was raised against a synthetic peptide containing dimethyl Lys4 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: 5 µg/ml

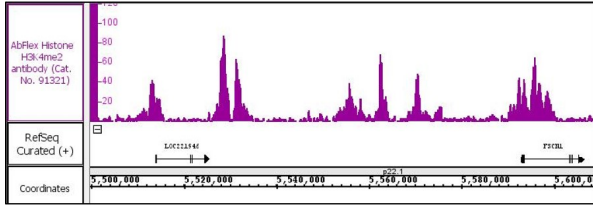
WB\*: 0.5 - 2 µg/ml

DB: 1 µg/ml

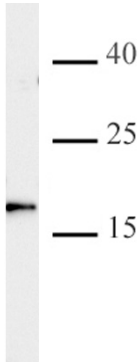
\*Note: many chromatin-bound proteins are not soluble in a low salt nuclear extract and fractionate to the pellet. Therefore, we recommend a High Salt / Sonication Protocol when preparing nuclear extracts for Western Blot.

**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.

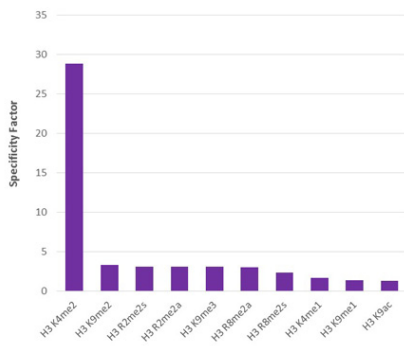


AbFlex® Histone H3K4me2 recombinant antibody (rAb) tested by ChIP-Seq  
 Chromatin immunoprecipitation (ChIP) was performed using the ChIP-IT® High Sensitivity Kit (Cat. No. 53040) with 30 µg of HeLa cell chromatin and 5 µg of AbFlex Histone H3K4me2 antibody. ChIP DNA was sequenced on the Illumina NextSeq and 14.2 million sequence tags were mapped to identify Histone H3K4me2 binding sites on chromosome 7.



### AbFlex® Histone H3K4me2 (rAb) tested by Western blot.

Detection of H3K4me2 by Western blot. The analysis was performed using 30 µg HeLa nuclear extract and AbFlex® Histone H3K4me2 (rAb) at a 2 µg/mL dilution.



### AbFlex® Histone H3K4me2 specificity tested by peptide array analysis.

Peptide array analysis was used to confirm the specificity of this antibody for its intended modification. Histone H3K4me2 antibody was applied 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 and those related to the immunogen.