

## Histone H3K9me3 antibody (pAb)

**Catalog Nos:** 39765, 39065, 39766

**RRID:** AB\_2793334

**Isotype:** IgG

**Application(s):** ChIP, CUT&Tag, DB, ICC, IF, WB

**Reactivity:** Human, Wide Range Predicted

**Quantities:** 100 µg, 50 µg, 10 µg

**Purification:** Protein A Chromatography

**Host:** Rabbit

**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 9 of histone H3 can be mono-, di- or trimethylated by different histone methyltransferases (HMTs) such as SuvH39H1 or G9a. This methylated lysine can be demethylated by histone demethylases as JMJD1A, LSD1 or JMJD2C. Methylation of this residue is mainly associated with transcriptional repression.

**Immunogen:** This Histone H3 trimethyl Lys9 antibody was raised against a peptide including trimethyl-lysine 9 of histone H3.

**Buffer:** Purified IgG in PBS with 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic. For your convenience, an unpurified serum version (Catalog No. 39161) of this antibody is also available.

### Application Notes:

Applications Validated by Active Motif:

ChIP: 10 µg per ChIP

ICC/IF: 1 µg/ml dilution

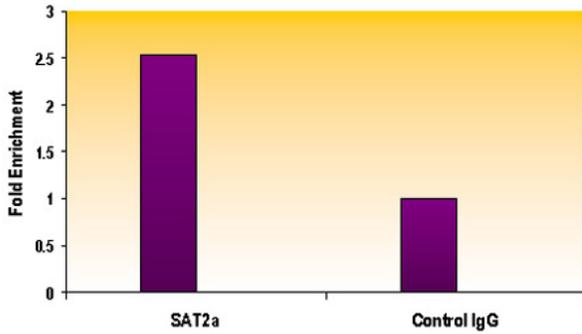
WB\*: 0.5 - 2 µg/ml dilution

CUT&Tag: 1 µg 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.

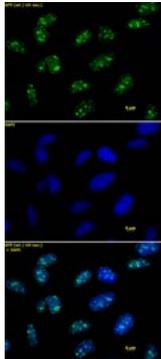
**Storage and Guarantee:** Some products may be shipped at room temperature. This will not affect their stability or performance. Avoid repeated freeze/thaw cycles by aliquoting items into single-use fractions for storage at -20°C for up to 2 years. Keep all reagents on ice when not in storage. This 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 H3 trimethyl Lys9 antibody tested by ChIP analysis.

Chromatin IP performed using the ChIP-IT<sup>®</sup> Express Kit (Catalog No. 53008) and HeLa Chromatin ( $1.5 \times 10^6$  cell equivalents per ChIP) using 10  $\mu\text{g}$  of Histone H3 trimethyl Lys9 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.

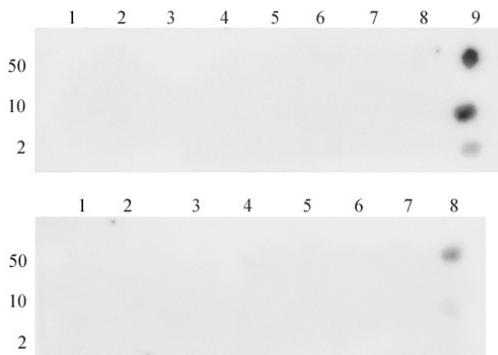
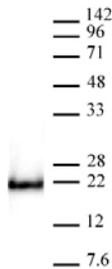


#### Histone H3 trimethyl Lys9 antibody tested by immunofluorescence.

Detection of Histone H3 trimethyl Lys9 by immunofluorescence. HeLa cells were stained with Histone H3 trimethyl Lys9 antibody at a dilution of 1  $\mu\text{g}/\text{ml}$ . Top panel: Histone H3 trimethyl Lys9 antibody staining. Middle panel: DAPI. Bottom panel: merge.

#### Histone H3K9me3 antibody (pAb) tested by Western blot.

HeLa nuclear extract (20  $\mu\text{g}$ ) was probed with Histone H3K9me3 antibody (2  $\mu\text{g}/\text{ml}$  dilution).



#### Histone H3K9me3 tested by dot blot analysis to confirm the specificity of Histone H3K9me3.

Peptides corresponding to regions around major sites of histone H3 methylation were spotted onto PVDF and probed with antibody at 2  $\mu\text{g}/\text{ml}$ . The amount of peptide (picomoles) spotted is indicated next to each row.

Top row Lane 1: unmodified Lys4. Lane 2: H3K4me1. Lane 3: H3K4me1. Lane 4: H3K4me2. Lane 5: H3K4me3. Lane 6: unmodified K9. Lane 7: H3K9me1. Lane 8: H3K9me2. Lane 9: H3K9me3.

Bottom row, Lane 1: unmodified K27. Lane 2: H3K27me1. Lane 3: H3K27me2. Lane 4: H3K27me3. Lane 5: Unmod K36. Lane 6: H3K36me1. Lane 7: H3K36me2. Lane 8: H3K36me3.