

## Histone H3K27me3 antibody (pAb)

**Catalog Nos:** 39155, 39055, 39157

**RRID:** AB\_2561020

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

**Reactivity:** Human, Monkey, Mouse, Wide Range Predicted

**Quantities:** 100 µg, 50 µg, 10 µg

**Purification:** Protein A Chromatography

**Host:** Rabbit

**Isotype:** IgG

**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; 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. Histone H3K27 can be mono-, di- or trimethylated by different histone methyltransferases, such as EZH2 or NSD3. While histone methylation can be associated with transcriptional activation or repression, methylation of Lysine 27 of histone H3 is mainly associated with transcriptional repression.

**Immunogen:** This antibody was raised against a peptide including trimethyl-lysine 27 of histone H3.

**Buffer:** Purified IgG in PBS (pH 7.5) with 30% glycerol and 0.035% sodium azide. Sodium azide is highly toxic. For your convenience, an unpurified serum version (Catalog No. 39156) of this antibody is also available.

### Application Notes:

Applications Validated by Active Motif:

ChIP: 5 - 10 µg per ChIP

ChIP-Seq: 5 µg each

ICC/IF: 2 µg/ml dilution

IHC(FFPE): 2 µg/ml dilution

WB\*: 0.5 - 2 µg/ml dilution

CUT&Tag: 1 µg per 50 µl reaction\*

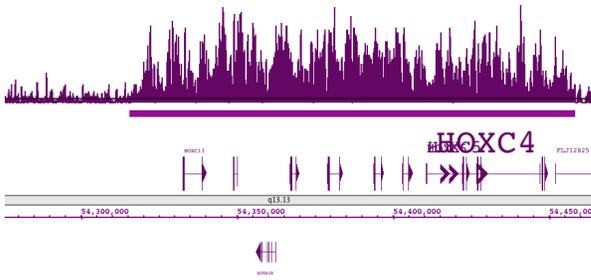
CUT&RUN: 1 µg per 50 µl reaction\*

\*This antibody has been validated for CUT&Tag using Active Motif's CUT&Tag-IT™ Assay Kit, Catalog No. 53160.

\*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 WB.

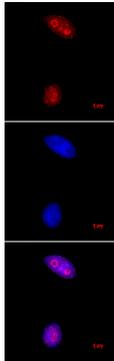
For H3K27me3, we also offer AbFlex® H3K27me3 Recombinant Antibody (rAb). For details, see Catalog No. 91167.

**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 H3K27me3 pAb tested by ChIP-Seq.

ChIP was performed using chromatin from the human iPS 19.11 cell line. ChIP DNA was sequenced on the Illumina GA II and sequence tags were mapped to identify H3K27me3 binding. The image shows H3K27me3 binding across a 140,000 bp region of the HOXC gene cluster on chromosome 12.



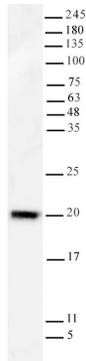
#### Histone H3K27me3 pAb tested by immunofluorescence.

HeLa cells stained with Histone H3 trimethyl Lys27 antibody (1.7 µg/ml dilution).

Top panel: Histone H3 trimethyl Lys27 antibody.

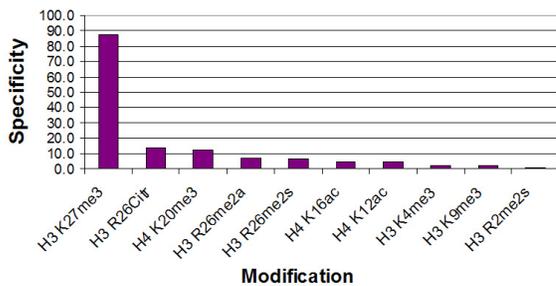
Middle: DAPI.

Bottom: Merge of both images.



#### Histone H3K27me3 pAb tested by Western blot.

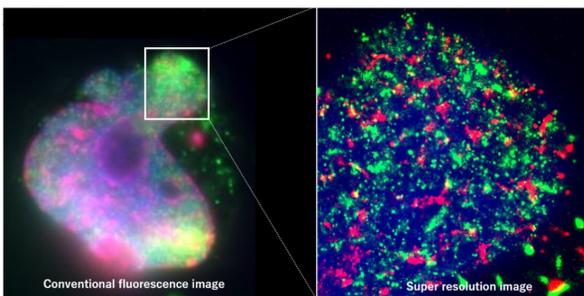
HeLa cell nuclear extract (20 µg) probed with Histone H3K27me3 pAb at 1 µg/ml.



#### Histone H3 trimethyl Lys27 antibody specificity tested by peptide array analysis.

Peptide array analysis was used to confirm the specificity of this antibody for its intended modification. Histone H3 trimethyl Lys27 antibody was applied at a dilution of 1:5,000 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. Recognition of the H3 trimethyl Lys27 peptides by the antibody blocked by phosphorylation at Ser28.

Array Data File



#### Histone H3K27me3 antibody tested by Sysmex Corporation using their Super Resolution Microscope HM-1000

HeLa cells were stained with Histone H3 trimethyl Lys27 pAb, with Janelia Fluor 646 (orange) conjugated anti-rabbit IgG as a secondary antibody.

Images were obtained at an exposure time of 30 milliseconds.