

Histone H3K9me1 Antibody, SNAP-Certified™ for CUT&RUN and ChIP



EpiCypher®

Catalog No 13-0029

Lot No 20281003-45

Pack Size 100 µg

Type Monoclonal **Host** Rabbit

Target Size 15 kDa

Format Aff. Pur. IgG

Reactivity Human, Mouse, Wide Range
(Predicted)

Applications CUT&RUN, ChIP, ICC, WB

Product Description:

This antibody meets EpiCypher's lot-specific SNAP-Certified™ criteria for specificity and efficient target enrichment in both CUT&RUN and ChIP applications. In CUT&RUN, this requires <20% cross-reactivity to related histone PTMs determined using the SNAP-CUTANA™ K-MetStat Panel of spike-in controls (EpiCypher 19-1002, Figure 1). High target efficiency is confirmed by consistent genomic enrichment at 500k and 50k starting cells (Figures 2-3). In ChIP, this requires <20% cross-reactivity to related histone PTMs and >5% of target input recovered as determined using the SNAP-ChIP® K-MetStat Panel of spike-in controls (EpiCypher 19-1001, Figure 6) [1].

Immunogen:

A synthetic peptide corresponding to histone H3 monomethylated at lysine 9.

Formulation:

Protein A affinity-purified antibody (1 mg/mL) in PBS, with 0.09% sodium azide, 1% BSA, and 50% glycerol.

Storage and Stability:

Stable for 1 year at -20°C from date of receipt.

Recommended Dilution:

CUT&RUN: 0.5 µg per reaction

ChIP: 2 - 5 µg per 5 µg chromatin

IF/ICC: 0.5 - 2 µg/mL

WB: 0.2 - 1 µg/mL

References:

[1] Shah et al (2018) Mol Cell 72:162

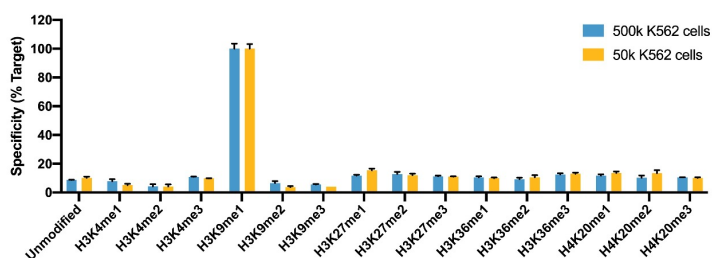


Figure 1: SNAP specificity analysis in CUT&RUN. CUT&RUN was performed on 500k and 50k native K562 cells with the SNAP-CUTANA K-MetStat Panel (EpiCypher 19-1002) spiked-in prior to the addition of 0.5 µg H3K9me1 antibody. CUT&RUN sequencing reads were aligned to the unique DNA barcodes corresponding to each nucleosome in the K-MetStat panel (x-axis). Data are expressed as a percent relative to on-target recovery (H3K9me1 set to 100%). Error bars represent mean ± SEM for the duplicate DNA barcodes in a single CUT&RUN experiment.

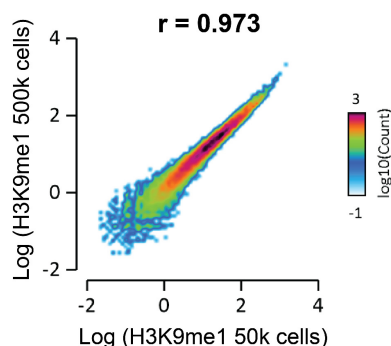


Figure 2: Antibody efficiency analysis in CUT&RUN using cell input correlation. Genome-wide correlation analysis was performed to compare H3K9me1 antibody enrichment in CUT&RUN using 500k and 50k cell inputs. The log of the number of reads per 75 bp binned region across the genome is plotted for both samples. CUT&RUN data generated using this H3K9me1 antibody are highly correlated between the two cell inputs (Pearson correlation $r = 0.973$), indicating high consistency of H3K9me1 antibody target recovery.

This product is for *in vitro* research use only and is not intended for use in humans or animals.

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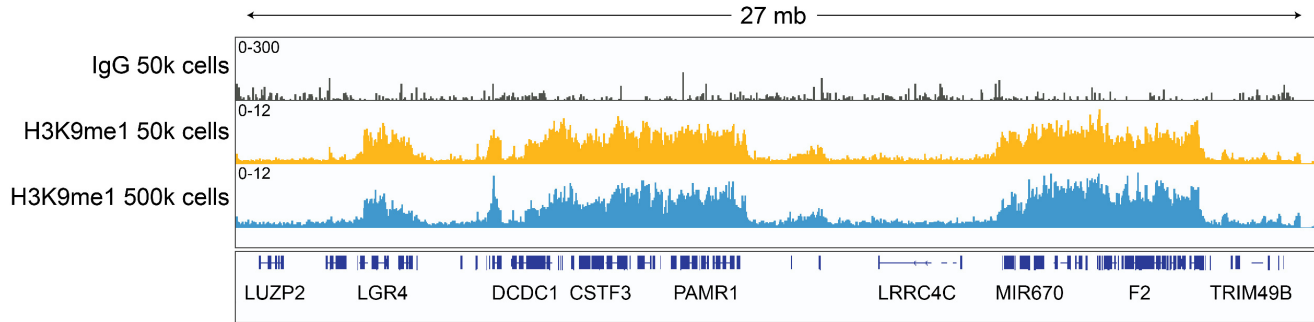


Figure 3: H3K9me1 CUT&RUN representative tracks at 500k and 50k cells. Gene browser shots generated using the Integrative Genomics Viewer (IGV, Broad Institute) show representative loci for H3K9me1 antibody tested in CUT&RUN using 500k and 50k cells. Similar results in peak structure and location were observed for both cell inputs. Methods: CUT&RUN was performed using the CUTANA™ ChIC/CUT&RUN Kit v2.0 (EpiCypher 14-1048) adapted to high throughput Tecan liquid handling. Library preparation was performed with 5 ng DNA using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina®. Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 4.5 million reads (IgG 50k cell input), 14.2 million reads (H3K9me1 50k cell input), and 10.1 million reads (H3K9me1 500k cell input).

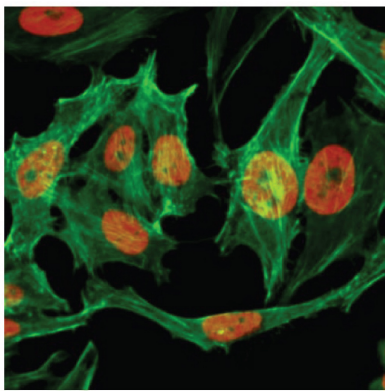


Figure 4: Immunocytochemistry: ICC of HeLa cells using 2 µg/mL of H3K9me1 antibody (red). Actin filaments were labeled with fluorescein phalloidin (green).



Figure 5: Western blot data: Recombinant histone H3.3 (Lane 1) and acid extracts of HeLa cells (Lane 2) were blotted onto PVDF and probed with 0.5 µg/mL H3K9me1 antibody.

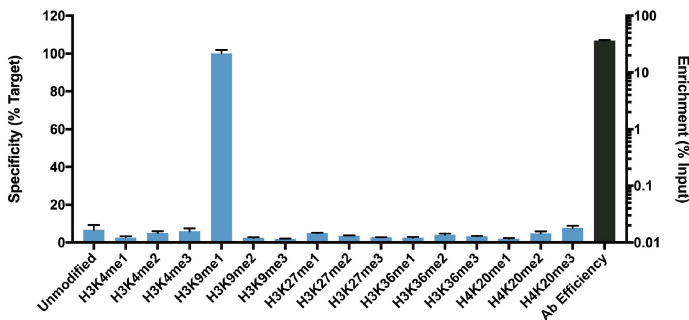


Figure 6: SNAP-ChIP-qPCR specificity and enrichment analysis. Histone H3K9me1 antibody (3 µg) was tested in a native ChIP experiment using chromatin from K562 cells (3 µg) with the SNAP-ChIP K-MetStat Panel (EpiCypher 19-1001) spiked-in prior to micrococcal nuclease digestion. Specificity (left y-axis) was determined by qPCR for the DNA barcodes corresponding to modified nucleosomes in the SNAP-ChIP panel (x-axis). Black bar represents antibody efficiency (right y-axis; log scale) and indicates percentage of the target immunoprecipitated relative to input. Error bars represent mean ± SEM in replicate ChIP experiments.

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