Histone H3K27me3 Antibody, SNAP-ChIP® Certified, CUTANA™ CUT&RUN Compatible

Catalog No. 13-0030
Lot No. 18303001
Pack Size 100 µg

Type	Monoclonal	**Target Size**	15 kDa	**Reactivity**	H, M, WR
Host	Rabbit	**Format**	Aff. Pur. IgG	**Appl.**	ChIP, ChIP-Seq, CUT&RUN, Luminex, WB, IHC, IF

Product Description:

This antibody meets EpiCypher's "SNAP-ChIP® Certified" criteria for specificity and target enrichment in ChIP (<20% cross-reactivity to related histone post-translational modifications and >5% recovery of target input determined using SNAP-ChIP K-MetStat Panel spike-in controls; EpiCypher Catalog No. 19-1001). Although its specificity in CUT&RUN has yet to be empirically determined *in situ* using spike-in controls, CUT&RUN data produced by this antibody shows a genome wide enrichment pattern characteristic of H3K27me3 and is highly correlated with ChIP-seq (Figures 3-5).

Immunogen:

A synthetic peptide corresponding to histone H3 trimethylated at lysine 27.

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.

Application Notes:

Recommended Dilutions:

ChIP / ChIP-seq: 2 - 5 µg per 5 µg chromatin
CUT&RUN: 1:100 **Luminex**: 1:4000
WB: 1 - 2 µg/mL **IHC / IF**: 0.5 - 2 µg/mL

References:

Grzybowski et al (2015) Mol Cell 58:886
Shah et al (2018) Mol Cell 72:162

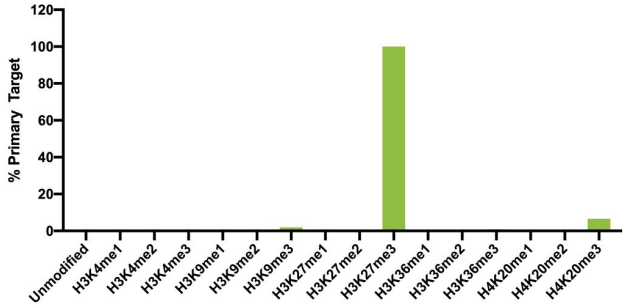

Figure 1: Luminex multiplexed specificity profiling. H3K27me3 antibody was assessed using a Luminex® based approach employing dCypher® Nucleosome K-MetStat Panel (EpiCypher Catalog No. 16-9002). The panel comprises biotinylated designer nucleosomes (x-axis) individually coupled to color coded Luminex Magplex® beads. Antibody binding to the panel of 16 nucleosomes was tested in multiplex at a 1:4000 dilution, and detected with second layer anti-IgG*PE. Data was generated using a Luminex FlexMAP3D®. Data is normalized to target (H3K27me3; set to 100).

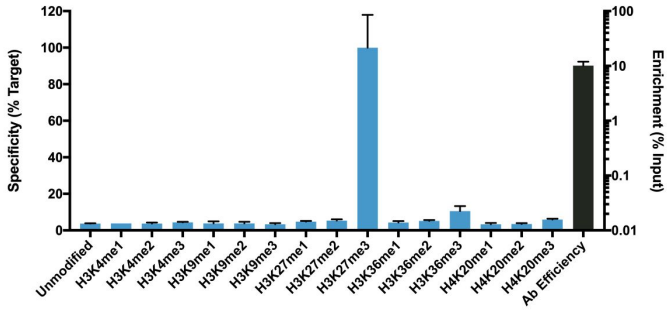

Figure 2: SNAP-ChIP-qPCR specificity and enrichment analysis. H3K27me3 antibody (5 µg) was tested in a native ChIP experiment using chromatin from K-562 cells (5 µg) with the SNAP-ChIP K-MetStat Panel (EpiCypher Catalog No. 19-1001) spiked-in prior to micrococcal nuclease digestion. Specificity (left 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.

Applications Key: ChIP: Chromatin immunoprecipitation; ChIP-seq: ChIP-sequencing; CUT&RUN: Cleavage Under Targets and Release Using Nuclease; WB: Western Blot; L: Luminex; E: ELISA; FACS: Flow cytometry; IP: Immunoprecipitation; IF: Immunofluorescence; IHC: Immunohistochemistry; ICC: Immunocytochemistry. **Reactivity Key:** B: Bovine; Ce: *C. elegans*; Ch: Chicken; Dm: *Drosophila*; Eu: Eukaryote; H: Human; M: Mouse; Ma: Mammal; R: Rat; Sc: *S.cerevesiae*; Sp: *S. pombe*; WR: Wide Range (predicted); X: Xenopus; Z: Zebrafish

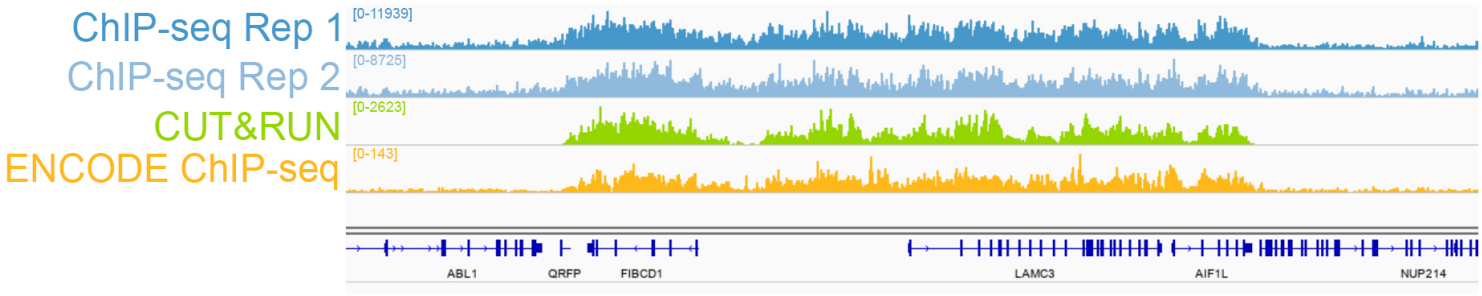

Figure 3: H3K27me3 SNAP-ChIP-seq and CUT&RUN representative tracks. A gene browser shot generated using the Integrative Genomics Viewer (IGV, Broad Institute) shows a representative locus for EpiCypher H3K27me3 ChIP-seq replicate experiments (blue tracks, 5 µg antibody) and CUT&RUN (green track, 1:100 antibody dilution). For comparison ENCODE H3K27me3 ChIP-seq using a different antibody is shown (bottom orange track, GEO accession number GSM733658). Similar results in peak structure and location were observed throughout the genome for EpiCypher H3K27me3 antibody in ChIP-seq and CUT&RUN. *Methods: Native ChIP-seq was performed as described (Shah et al., Mol Cell 2018). CUT&RUN was performed using EpiCypher CUTANA pAG-MNase for ChIC/CUT&RUN (EpiCypher Catalog No. 15-1016) as described (EpiCypher.com/cutana-protocol). Library preparation was performed with 10 ng DNA using the NEBNext® Ultra™ II DNA Library Prep Kit for Illumina®. ChIP libraries were sequenced on an Illumina NextSeq 550 (2x150bp paired end). The total number of reads was 37.8 and 34.9 million for the ChIP-seq replicates and 8.0 million for CUT&RUN.*

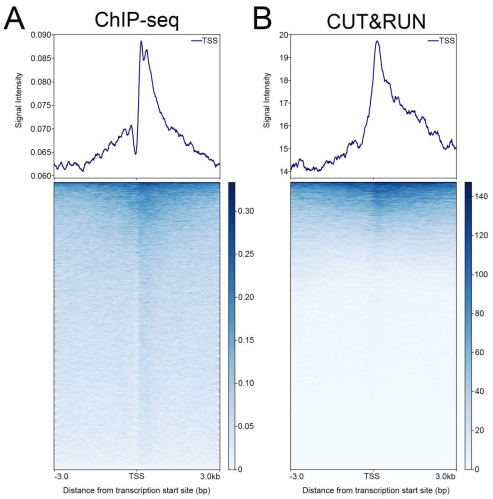

Figure 4: ChIP-seq and CUT&RUN genome wide analysis. EpiCypher H3K27me3 antibody was tested in native ChIP-seq (**A**) and CUT&RUN (**B**) using the methods described above. Genome-wide analysis of H3K27me3 enrichment (signal intensity) flanking annotated transcription start sites (TSSs; +/- 3kb) is graphed as a cumulative histogram plot (top) and shown in a heatmap (bottom). Individual gene loci in each row of the heatmap are colored by signal intensity and sorted by strongest to lowest enrichment (top to bottom). EpiCypher H3K27me3 antibody displays a characteristic enrichment pattern downstream of the TSS, remaining elevated throughout the body of the gene.

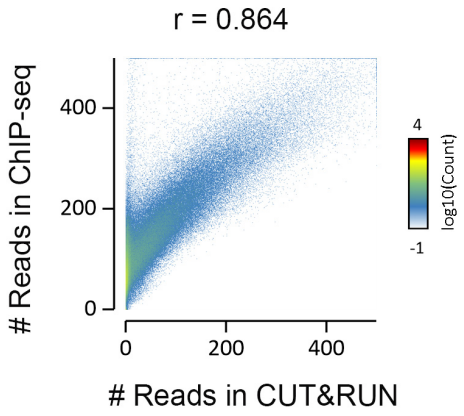

Figure 5: ChIP-seq vs. CUT&RUN correlation analysis. Genome-wide correlation analysis was performed to compare EpiCypher H3K27me3 antibody enrichment in ChIP-seq and CUT&RUN. The number of reads per 10 kb binned region across the genome is plotted for CUT&RUN (x-axis) vs. ChIP-seq (y-axis) (EaSeq). ChIP-seq and CUT&RUN data generated using this antibody are highly correlated (Pearson correlation r = 0.864).

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Figure 6: Western Blot analysis. Recombinant histone H3.3 (Lane 1) and acid extracts of HeLa cells (Lane 2) were blotted onto PVDF and probed with 1 µg/mL EpiCypher H3K27me3 Antibody.

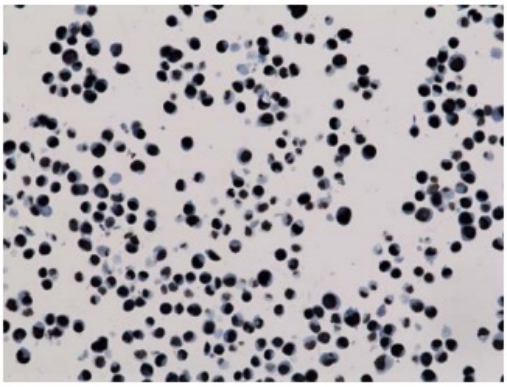

Figure 7: Immunohistochemistry (IHC). IHC staining of HepG2 cells using EpiCypher H3K27me3 Antibody.

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