H3K27ac Antibody, SNAP-Certified™ for CUT&RUN and CUT&Tag

Catalog No	13-0059	**Type**	Monoclonal [2114-3E4]
Lot No	24264002-84	**Host**	Rabbit
Pack Size	100 µg	**Concentration**	0.5 mg/mL
Applications	CUT&RUN, CUT&Tag	**Reactivity**	Human, Wide Range (Predicted)

DESCRIPTION

This H3K27ac (histone H3 acetylated at lysine 27) antibody meets EpiCypher's SNAP-Certified™ criteria for specificity and efficient target enrichment in both CUT&RUN and CUT&Tag applications. This requires <20% cross-reactivity to related histone PTMs determined using the SNAP-CUTANA™ K-AcylStat Panel of spike-in controls (EpiCypher RD193002, **Figures 1 and 4**). High target efficiency is confirmed by consistent genomic enrichment at varying cell inputs: 500k and 50k cells in CUT&RUN (**Figures 2-3**); 100k and 10k cells in CUT&Tag (**Figures 5-6**). High efficiency antibodies display similar peak structures at representative loci (**Figures 3 and 6**) and highly conserved genome-wide signal (**Figures 2 and 5**) even at reduced cell numbers. H3K27ac is associated with gene activation and is enriched at active enhancers and promoters [1].

TECHNICAL INFORMATION

Immunogen	A synthetic peptide corresponding to histone H3 acetylated at lysine 27
Storage	Stable for 1 year at 4°C from date of receipt
Formulation	Protein A affinity-purified recombinant monoclonal antibody in borate buffered saline pH 8.0, 0.09% sodium azide
Target Size	15 kDa

RECOMMENDED DILUTION

CUT&RUN:	0.5 µg per reaction	**CUT&Tag:**	0.5 µg per reaction

GENE & PROTEIN INFORMATION

Uniprot ID	H3.1 - P68431
Alternate Names	H3, H3/a, H3/b, H3/c, H3/d

REFERENCES

[1] Pei et al. *Clin. Epigenetics* (2020). PMID: 32664951
[2] Simithy et al. *Nat. Commun.* (2017). PMID: 29070843

VALIDATION DATA

CUT&RUN Methods

CUT&RUN was performed on 500k and 50k K562 cells with the SNAP-CUTANA™ K-MetStat Panel (EpiCypher 19-1002) or SNAP-CUTANA™ K-AcylStat Panel (EpiCypher RD193002) spiked-in prior to the addition of 0.5 µg of either IgG negative control (EpiCypher 13-0042), H3K4me3 positive control (EpiCypher 13-0060), or H3K27ac antibodies. The experiment was performed using the CUTANA™ ChIC/CUT&RUN Kit v4 (EpiCypher 14-1048). Library preparation was performed with 5 ng of CUT&RUN enriched DNA (or the total amount recovered if less than 5 ng) using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001/14-1002). Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 9.4 million reads (IgG 500k cell input), 12.0 million reads (IgG 50k cell input), 12.3 million reads (H3K4me3 500k cell input), 9.4 million reads (H3K4me3 50k cell input), 13.0 million reads (H3K27ac 500k cell input), and 8.4 million reads (H3K27ac 50k cell input). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

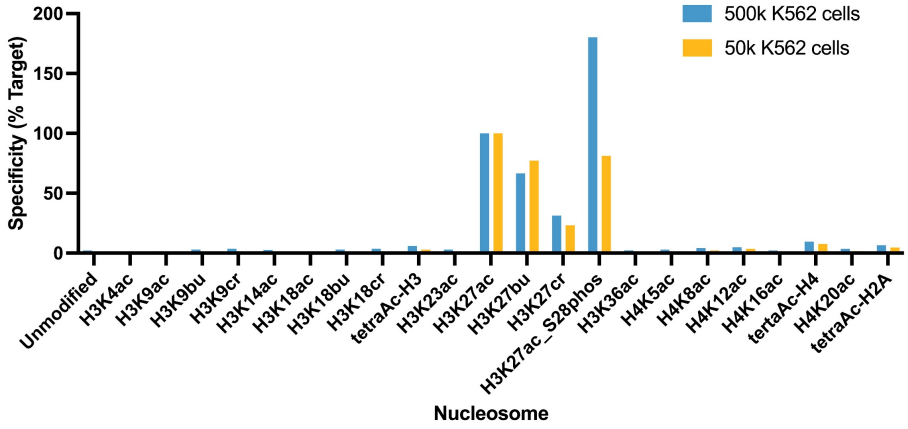

FIGURE 1 SNAP specificity analysis in CUT&RUN. CUT&RUN was performed as described above. CUT&RUN sequencing reads were aligned to the unique DNA barcodes corresponding to each nucleosome in the K-AcylStat panel (x-axis). Data are expressed as a percent relative to on-target recovery (H3K27ac set to 100%). The antibody showed recovery of H3K27ac spike-in nucleosomes as well as H3K27ac nucleosomes that contain a proximal phosphorylation at S28 at both 500k and 50k cells. The antibody cross-reacts with extended acyl states (butyrylation and crotonylation) at H3K27, but these are typically low abundance in

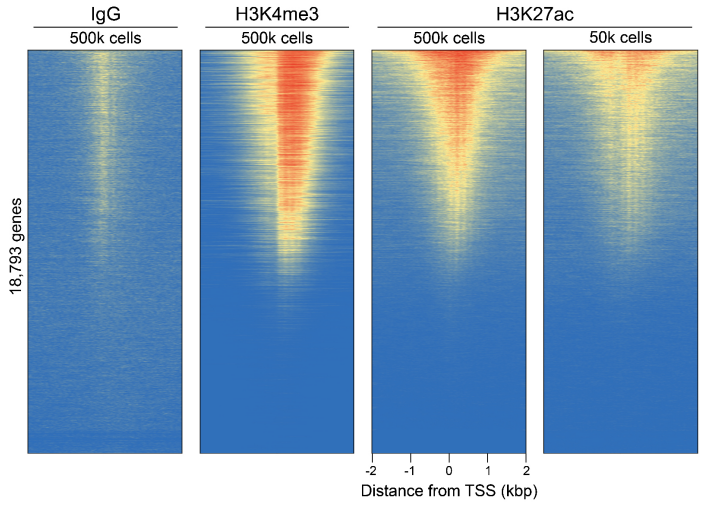

FIGURE 2 CUT&RUN genome-wide enrichment. CUT&RUN was performed as described above. Sequence reads were aligned to 18,793 annotated transcription start sites (TSSs ± 2 kbp). Signal enrichment was sorted from highest to lowest (top to bottom) relative to the H3K27ac - 500k cells reaction (all gene rows aligned). High, medium, and low intensity are shown in red, yellow, and blue, respectively. H3K4me3 positive control and H3K27ac antibodies produced the expected enrichment pattern, which was consistent between 500k and 50k cells and greater than the IgG negative control.

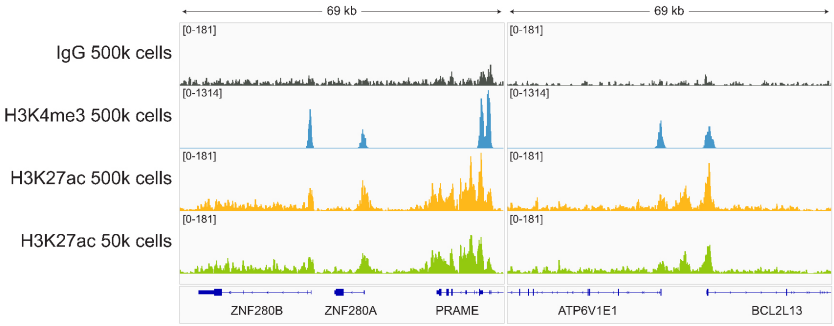

FIGURE 3 H3K27ac CUT&RUN representative browser tracks. CUT&RUN was performed as described above. Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). H3K27ac antibody tracks display peaks at promoters and enhancers, consistent with the biological function of this PTM. Similar results in peak structure and location were observed for both 500k and 50k cell inputs.

VALIDATION DATA

CUT&Tag Methods

CUT&Tag was performed on 100k and 10k K562 nuclei with the SNAP-CUTANA™ K-MetStat Panel (EpiCypher 19-1002) or SNAP-CUTANA™ K-AcylStat Panel (EpiCypher RD193002) spiked-in prior to the addition of 0.5 µg of either IgG negative control (EpiCypher 13-0042), H3K4me3 positive control (EpiCypher 13-0041), or H3K27ac antibodies. The experiment was performed using the CUTANA™ CUT&Tag Kit v1 (EpiCypher 14-1102/14-1103). Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 1.3 million reads (IgG 100k nuclei input), 2.0 million reads (IgG 10k nuclei input), 3.5 million reads (H3K4me3 100k nuclei input), 8.0 million reads (H3K4me3 10k nuclei input), 8.1 million reads (H3K27ac 100k nuclei input) and 8.5 million reads (H3K27ac 10k nuclei input). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

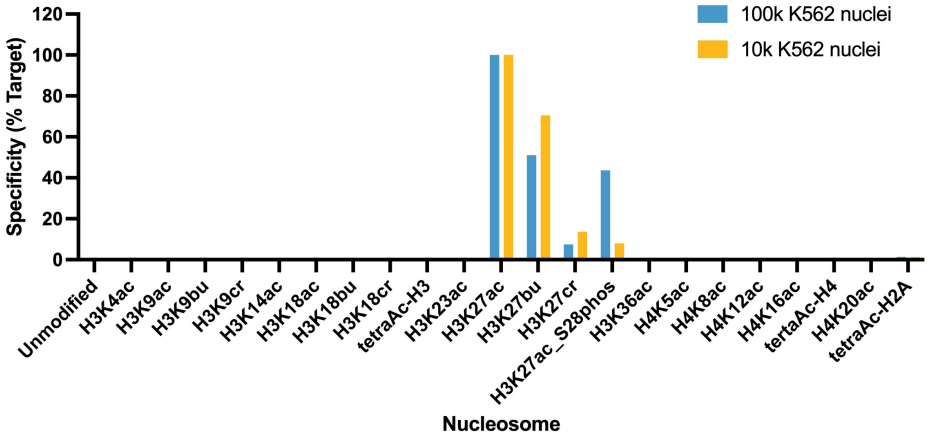

FIGURE 4 SNAP specificity analysis in CUT&Tag. CUT&Tag was performed as described above. CUT&Tag sequencing reads were aligned to the unique DNA barcodes corresponding to each nucleosome in the K-AcylStat panel (x-axis). Data are expressed as a percent relative to on-target recovery (H3K27ac set to 100%). The antibody showed recovery of H3K27ac spike-in nucleosomes and to a lesser extent H3K27ac nucleosomes that contain a proximal phosphorylation at S28 at both 500k and 50k cells. The antibody cross-reacts with butyrylation at H3K27, but this is typically low abundance in cells [2]. Data shown are representative (not lot-specific).

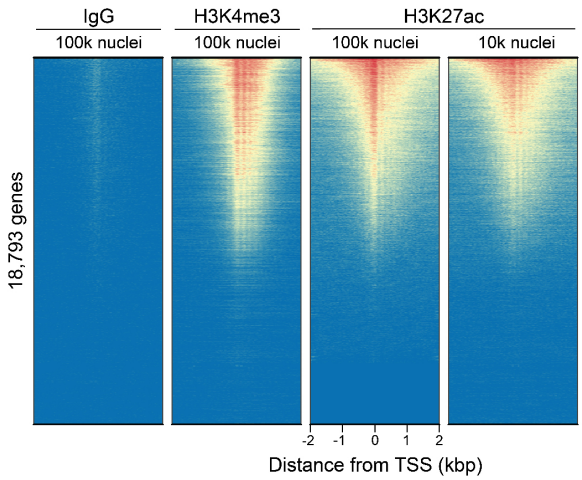

FIGURE 5 CUT&Tag genome-wide enrichment. CUT&Tag was performed as described above. Sequence reads were aligned to 18,793 annotated transcription start sites (TSSs ± 2 kbp). Signal enrichment was sorted from highest to lowest (top to bottom) relative to the H3K27ac - 100k nuclei reaction (all gene rows aligned). High, medium, and low intensity are shown in red, yellow, and blue, respectively. H3K4me3 positive control and H3K27ac antibodies produced the expected enrichment pattern, which was consistent between 100k and 10k nuclei and greater than the IgG negative control. Data shown are representative (not lot-specific).

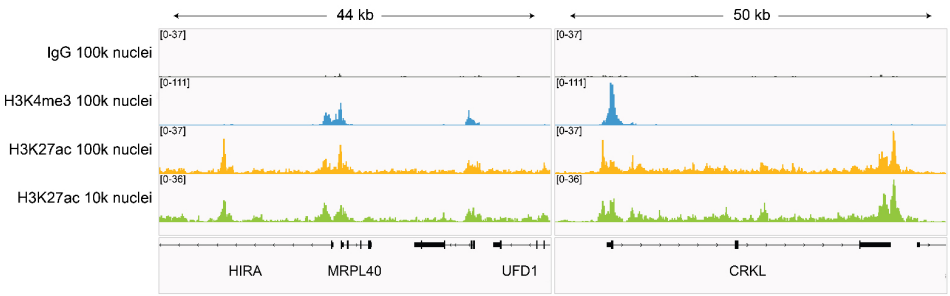

FIGURE 6 H3K27ac CUT&Tag representative browser tracks. CUT&Tag was performed as described above. Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). H3K27ac antibody tracks display peaks at promoters and enhancers, consistent with the biological function of this PTM. Similar results in peak structure and location were observed for both 100k and 10k nuclei inputs. Data shown are representative (not lot-specific).