Histone H3K4me2 Antibody, SNAP-Certified™ for CUT&RUN

Catalog No	13-0027	**Type**	Monoclonal
Lot No	23003005-81	**Host**	Rabbit
Pack Size	100 µg	**Concentration**	1 mg/mL
Applications	CUT&RUN, ICC, WB	**Reactivity**	Human, Mouse, Wide Range (Predicted)

DESCRIPTION

This antibody meets EpiCypher's lot-specific SNAP-Certified™ criteria for specificity and efficient target enrichment 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 2-4**). This antibody targets histone H3 dimethylated at lysine 4, which is enriched in promoters of transcriptionally active genes and genes primed for expression during cell development [1].

TECHNICAL INFORMATION

Immunogen	A synthetic peptide corresponding to histone H3 dimethylated at lysine 4
Storage	Stable for 1 year at -20°C from date of receipt
Formulation	Protein A affinity-purified antibody in PBS, 0.09% sodium azide, 1% BSA, and 50% glycerol
Target Size	15 kDa

RECOMMENDED DILUTION

CUT&RUN	0.5 µg per reaction
Immunocytochemistry	1 - 2 µg/mL
Western Blot	0.02 - 0.2 µg/mL

REFERENCES

[1] Pekowkska et al. *Genome Res.* (2010). PMID: 20841431

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) spiked-in prior to the addition of 0.5 µg of either H3K4me2 or IgG negative control (EpiCypher 13-0042) antibodies. The experiment was performed using the CUTANA™ ChIC/CUT&RUN Kit v2.0 (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). Both kit protocols were adapted for high throughput Tecan liquid handling. Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 3.0 million reads (IgG), 8.4 million reads (H3K4me2 500k cell input) and 5.7 million reads (H3K4me2 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.

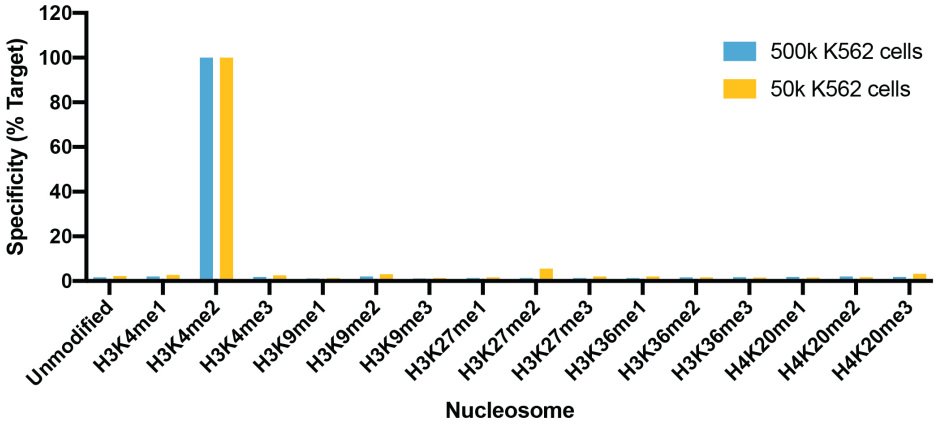

FIGURE 1 SNAP specificity analysis in CUT&RUN. CUT&RUN was performed as described above. CUT&RUN sequencing reads were aligned to the unique DNA barcodes corresponding to each nucleosome in the K-MetStat panel (x-axis). Data are expressed as a percent relative to on-target recovery (H3K4me2 set to 100%).

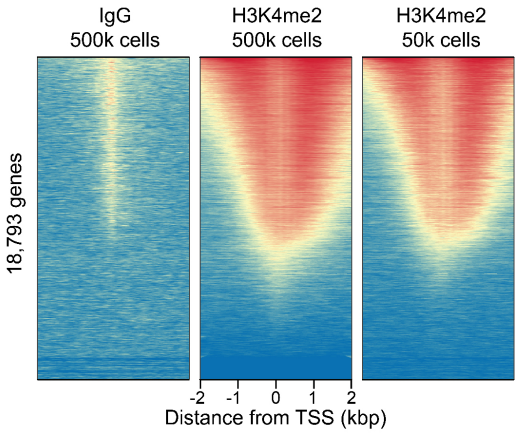

FIGURE 2 CUT&RUN genome wide enrichment. CUT&RUN was performed as described above. Sequence reads were aligned to 18.793 annotated transcription start sites (TSSs ± 2 kbp). Signal enrichment was sorted from highest to lowest (top to bottom) relative to the H3K4me2 - 50k cells sample (all gene rows aligned). High, medium, and low intensity are shown in red, yellow, and blue, respectively. H3K4me2 antibody produced the expected TSS enrichment pattern, which was consistent between 500k and 50k cells and greater than the IgG negative control.

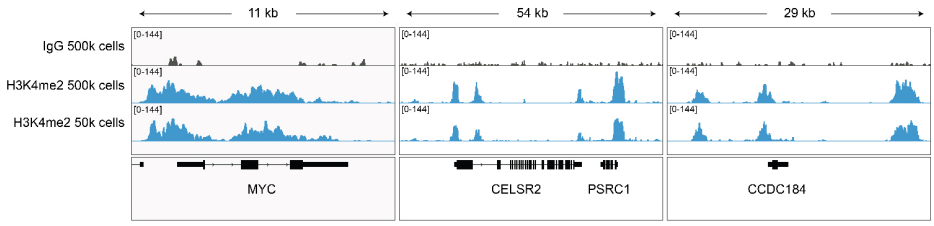

FIGURE 3 H3K4me2 CUT&RUN representative browser tracks. CUT&RUN was performed as described above. Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). Similar results in peak structure and location were observed for both cell inputs.

VALIDATION DATA

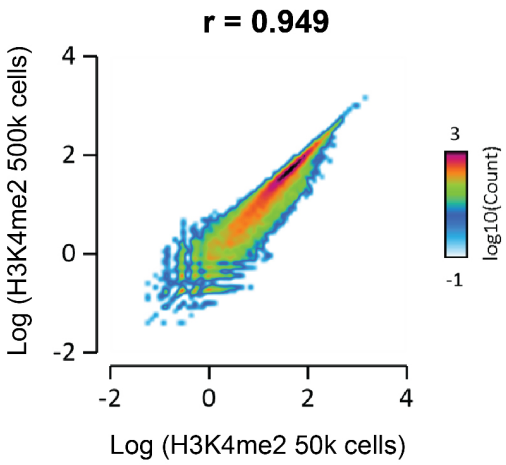

FIGURE 4 **Antibody efficiency analysis in CUT&RUN using cell input correlation.** CUT&RUN was performed as described above. Genome-wide correlation analysis was performed to compare H3K4me2 antibody enrichment using 500k and 50k cell inputs. The log of the number of reads per 75 bp binned region across the genome is plotted for both samples. CUT&RUN data generated using this H3K4me2 antibody are highly correlated between the two cell inputs (Pearson correlation r = 0.949), indicating high efficiency of H3K4me2 antibody target recovery.

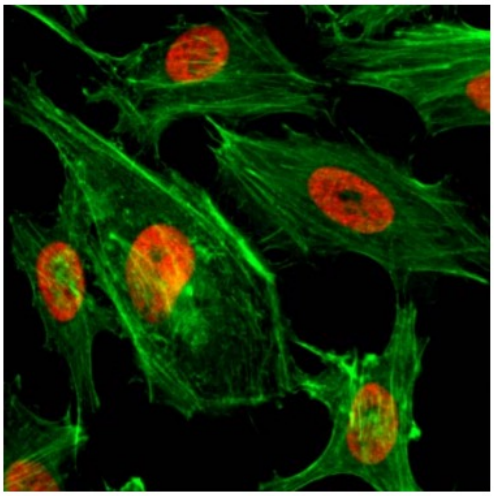

FIGURE 5 **Immunocytochemistry**. ICC of HeLa cells using 2 µg/mL of H3K4me2 antibody (red). Actin filaments were labeled with fluorescein phalloidin (green).

FIGURE 6 **Western blot data**. Recombinant histone H3.3 (Lane 1) and acid extracts of HeLa cells (Lane 2) were blotted onto PVDF and probed with 0.025 µg/mL of H3K4me2 antibody.