H4K20me3 Antibody, SNAP-Certified™ for CUT&RUN

Catalog No	13-0054	**Type**	Monoclonal
Lot No	23261002-81	**Host**	Mouse
Pack Size	100 µg	**Concentration**	1 mg/mL
Applications	CUT&RUN, WB	**Reactivity**	Human, *C. elegans*

DESCRIPTION

This H4K20me3 (histone H4 lysine 20 trimethyl) 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 2-3**).

TECHNICAL INFORMATION

Immunogen	A synthetic peptide corresponding to histone H4 trimethylated at lysine 20
Storage	Stable for 1 year at -20°C from date of receipt
Formulation	Protein A affinity-purified antibody in PBS, 0.1% sodium azide
Target Size	11.4 kDa

RECOMMENDED DILUTION

CUT&RUN:	0.5 µg per reaction
Western Blot:	1:4,000

GENE & PROTEIN INFORMATION

Uniprot ID	H4 - P62805

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 H4K20me3 or IgG negative control (EpiCypher 13-0042) antibodies. The experiment was performed using the CUTANA™ ChIC/CUT&RUN Kit v3 (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). Reaction sequencing depth was 4.6 million reads (IgG 50k cell input), 5.7 million reads (H4K20me3 500k cell input), and 10.4 million reads (H4K20me3 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.

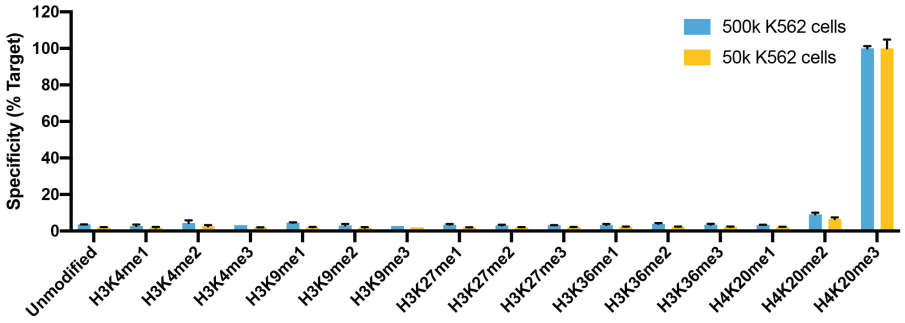

FIGURE 1 SNAP specificity analysis in CUT&RUN. CUT&RUN was performed as described above. CUT&RUN sequencing reads were aligned to the unique DNA barcodes corresponding to each nucleosome in the K-MetStat panel (x-axis). Data are expressed as a percent relative to on-target recovery (H4K20me3 set to 100%).

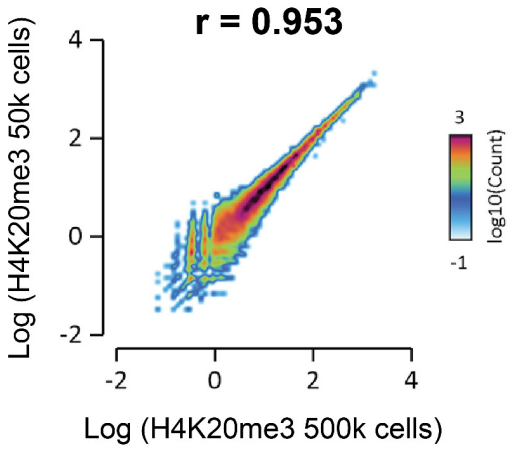

FIGURE 2 Antibody efficiency analysis in CUT&RUN using cell input correlation. Genome-wide correlation analysis was performed to compare H4K20me3 antibody enrichment in CUT&RUN using 500k cell and 50k cell inputs. The log of the number of reads per 75 bp binned region across the genome is plotted for both samples. CUT&RUN data generated using this H4K20me3 antibody are highly correlated between the two cell inputs (Pearson correlation r = 0.953), indicating high efficiency of H4K20me3 antibody target recovery.

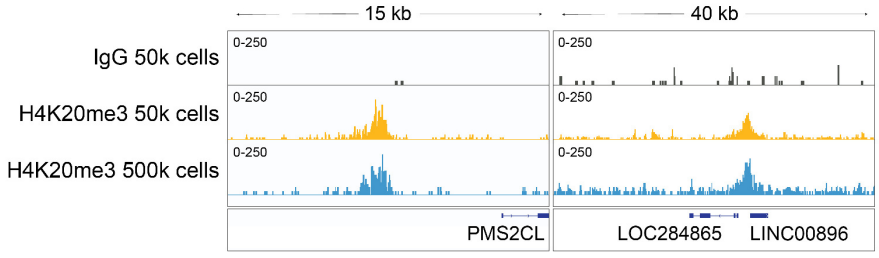

FIGURE 3 H4K20me3 CUT&RUN representative browser tracks. CUT&RUN was performed as described above. Gene browser shots generated using the Integrative Genomics Viewer (IGV, Broad Institute), Similar results in peak structure and location were observed for both cell inputs.

VALIDATION DATA

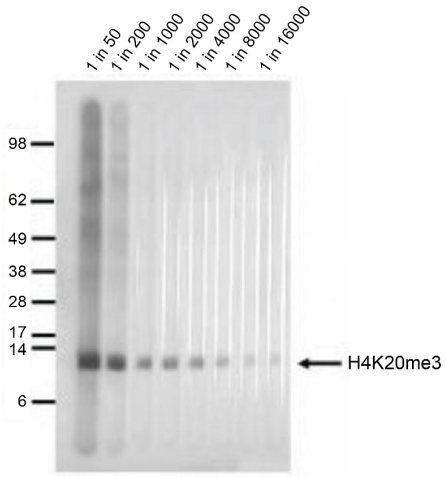

FIGURE 4 Western blot data. Representative Western data of H4K20me3 in HeLa cell lysates using H4K20me3 antibody at various dilutions.