H3K4me3 Antibody, SNAP-Certified™ for CUT&RUN

Catalog No	13-0041	**Type**	Mixed Monoclonal*
Lot No	23261009-82	**Host**	Rabbit
Pack Size	100 µg	**Concentration**	0.5 mg/mL
Applications	CUT&RUN, ICC/IF, WB	**Reactivity**	Human, Mouse, Drosophila, Yeast, Wide Range

DESCRIPTION

This H3K4me3 (histone H3 lysine 4 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 2-4**). This antibody targets histone H3 trimethylated at lysine 4, which is enriched at active promoters near transcription start sites (TSS).
**Mixed Monoclonal: a pool of multiple monoclonal antibodies.*

TECHNICAL INFORMATION

Immunogen	A synthetic peptide corresponding to histone H3 trimethylated at lysine 4
Storage	Stable for 1 year at -20°C from date of receipt
Formulation	Protein A affinity-purified antibody in PBS pH 7.4, 0.09% sodium azide
Target Size	15 kDa

RECOMMENDED DILUTION

CUT&RUN:	0.5 µg per reaction	**Western Blot:**	1:250
Immunofluorescence:	1:100		

GENE & PROTEIN INFORMATION

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

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 H3K4me3 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 8.3 million reads (IgG 500k cell input), 15.5 million reads (IgG 50k cell input), 9.8 million reads (H3K4me3 500k cell input) and 8.6 million reads (H3K4me3 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.

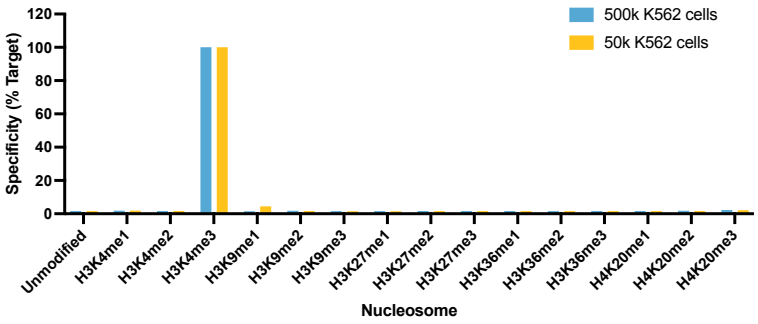

FIGURE 1 SNAP specificity analysis in CUT&RUN. CUT&RUN was performed as described above. CUT&RUN sequencing reads were aligned to the unique DNA barcodes corresponding to each nucleosome in the K-MetStat panel (x-axis). Data are expressed as a percent relative to on-target recovery (H3K4me3 set to 100%).

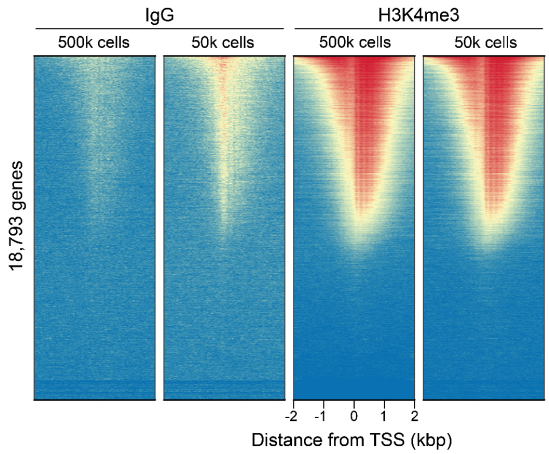

FIGURE 2 CUT&RUN genome wide enrichment. CUT&RUN was performed as described above. Sequence reads were aligned to 18,793 annotated transcription start sites (TSSs ± 2 kbp). Signal enrichment was sorted from highest to lowest (top to bottom) relative to the H3K4me3 - 500k cells sample (all gene rows aligned).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

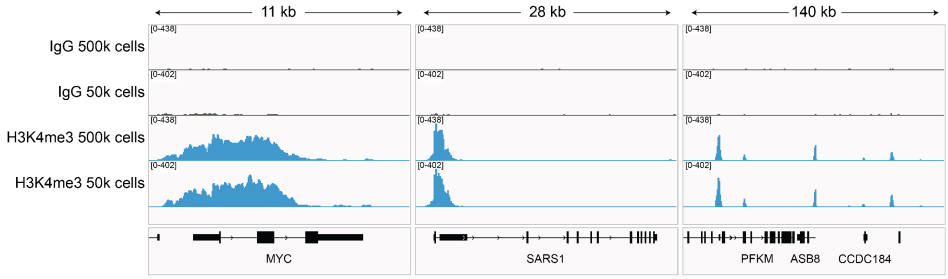

FIGURE 3 H3K4me3 CUT&RUN representative browser tracks. CUT&RUN was performed as described above. Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). Similar results in peak structure and location were observed for both cell inputs.

VALIDATION DATA

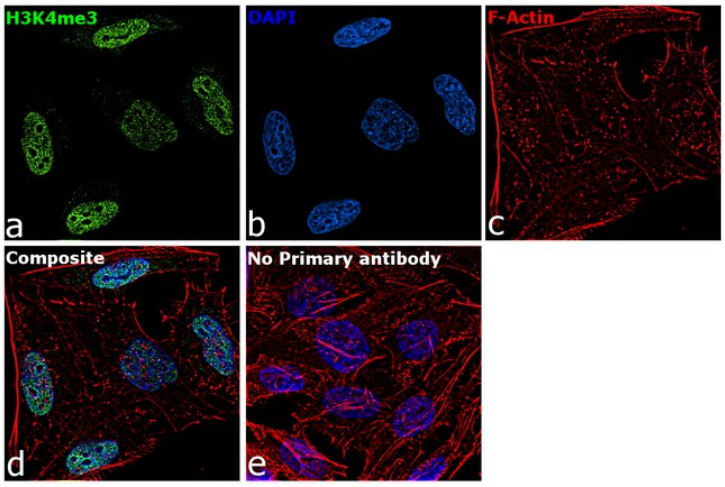

FIGURE 4 Immunofluorescence. Representative images (60X magnification) of HeLa cells fixed, permeabilized, and immunostained to show endogenous localization of H3K4me3. (**A**) H3K4me3 antibody (green, 1:100 dilution) detected with an Alexa Fluor® 488 conjugated anti-rabbit secondary. (**B**) DAPI stained nuclei (blue). (**C**) Rhodamine stained cytoskeletal F-actin (red). (**D**) A composite of panels a, b, & c demonstrating nuclear localization of H3K4me3. (**E**) Negative control lacking H3K4me3 primary antibody to assess background.

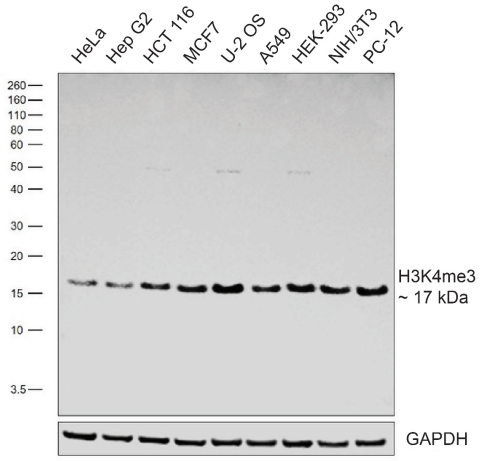

FIGURE 5 Western blot data. Representative Western data of H3K4me3 in whole cell extracts from HeLa, Hep G2, HCT 116, MCF7, U-2 OS, A549, HEK-293, NIH/3T3, and PC-12 cells. 30 µg of lysate was resolved via SDS-PAGE and detected with H3K4me3 antibody at a 1:250 dilution.