H3.3 Antibody, SNAP-Certified™ for CUT&RUN

Catalog No	13-0061	**Type**	Monoclonal [2893-4D5]
Lot No	24240001-83	**Host**	Rabbit
Pack Size	100 µg	**Concentration**	0.5 mg/mL
Applications	CUT&RUN	**Reactivity**	Human, Wide Range (Predicted)

DESCRIPTION

H3.3 Antibody, SNAP-Certified™ for CUT&RUN is an antibody to histone variant H3.3. H3.3 is enriched in promoters and enhancers of active genes as well as transcriptionally inactive heterochromatin, reflecting the diverse functions of this unique histone variant [1]. The antibody is highly specific for H3.3 without cross-reacting with canonical H3.1. Binding is not impacted by methylation or mutations at amino acids H3.3K27 or H3.3K36 (**Figure 1**).

H3.3 differs from canonical H3.1 at just five amino acids (31, 87, 89, 90, 96). Most of these changes are located in the inaccessible histone core, making them unfit epitopes for chromatin mapping experiments. The SNAP-Certified™ H3.3 antibody specifically interacts with amino acid 31, where a serine is substituted for alanine, allowing clear distinction between H3.3 and H3.1 in chromatin mapping experiments.

The H3.3 antibody meets EpiCypher's SNAP-Certified™ criteria for specificity and efficiency in CUT&RUN. This requires <20% cross-reactivity to other histone variants, determined using SNAP-CUTANA™ Spike-in Controls (**Figure 2**). High target efficiency is confirmed by consistent genomic enrichment at 500k and 50k cells (**Figure 3**).

Note: This antibody is sensitive to dithiothreitol (DTT).

TECHNICAL INFORMATION

Immunogen	A synthetic peptide corresponding to the N-terminal tail (including amino acid 31) of histone H3.3
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

Note: Not recommended for use with buffers containing reducing agents.

GENE & PROTEIN INFORMATION

UniProt ID H3.3 - P84243

REFERENCES

[1] Cohen & Meshorer *Trends Cell Biol.* (2024). PMID: 38614918

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) and Histone Variant Panel 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 H3.3 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). 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 5.2 million reads (IgG 500k cell input), 2.5 million reads (H3K4me3 500k cell input), 7.0 million reads (H3.3 500k cell input), and 3.7 million reads (H3.3 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.

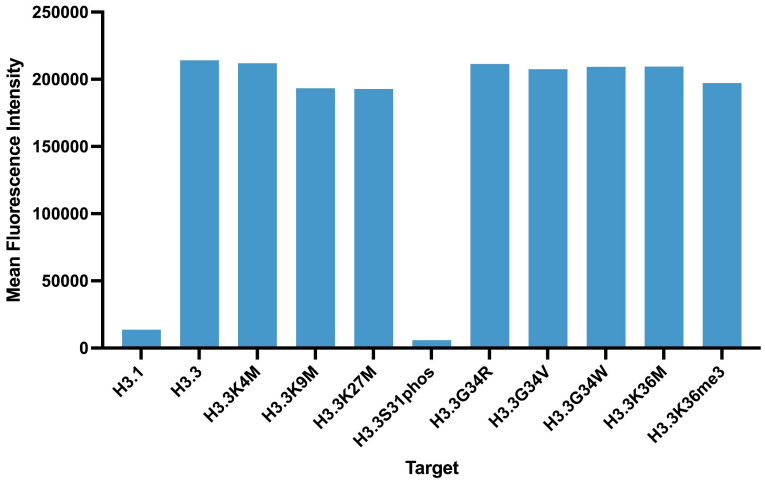

FIGURE 1 Luminex multiplexed specificity profiling. Histone H3.3 antibody was assessed using a Luminex® multiplexed binding assay. The panel of targets comprises biotinylated designer nucleosomes individually coupled to color-coded Luminex MagPlex® beads. Antibody binding to nucleosomes was tested in multiplex at a 1:250 dilution and detected with anti-IgG*Phycoerythrin (BioLegend 406421). Mean fluorescence intensity was quantified using Luminex FlexMAP 3D®. H3.3 antibody does not bind H3.1 and H3.3S31phos, as expected. H3.3 antibody binding is not affected by mutations or modifications of adjacent residues.

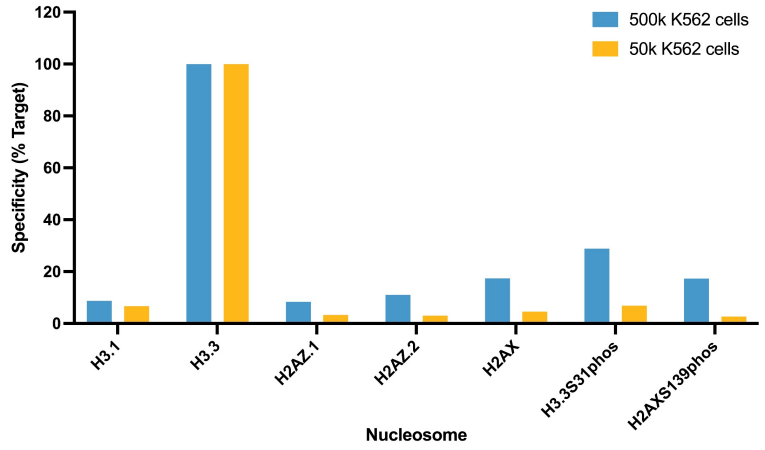

FIGURE 2 SNAP specificity analysis in CUT&RUN. CUT&RUN was performed as described above. CUT&RUN sequencing reads were aligned to the unique DNA barcodes corresponding to each nucleosome in the variant panel (x-axis). Data are expressed as a percent relative to on-target recovery (H3.3 set to 100%).

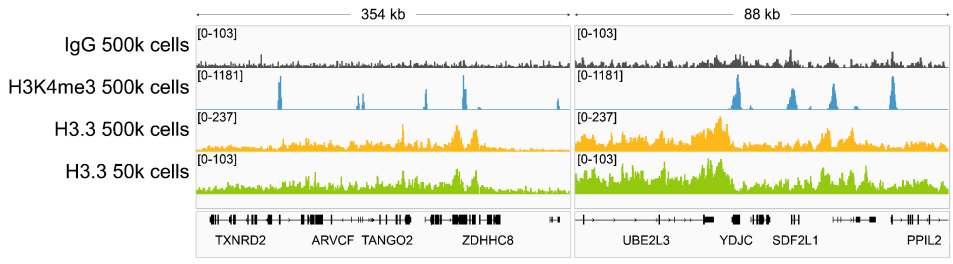

FIGURE 3 H3.3 CUT&RUN representative browser tracks. CUT&RUN was performed as described above. Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). Two representative loci of the top called peaks are shown. Similar results in peak structure and location were observed for both 500k and 50k cell inputs.