HA Tag CUTANA™ CUT&RUN Antibody

Catalog No	13-2010	**Type**	Polyclonal
Lot No	23332002-81	**Host**	Rabbit
Pack Size	100 µg	**Concentration**	1,000 µg/mL
Applications	CUT&RUN, WB	**Reactivity**	HA Epitope (YPYDVPDYA)

DESCRIPTION

This antibody meets EpiCypher's "CUTANA Compatible" criteria for performance in Cleavage Under Targets and Release Using Nuclease (CUT&RUN) and/or Cleavage Under Targets and Tagmentation (CUT&Tag) approaches to genomic mapping. Every lot of a CUTANA Compatible antibody is tested in the indicated approach using EpiCypher optimized protocols (epicypher.com/protocols) and determined to yield peaks that show a genomic distribution pattern consistent with reported function(s) of the target. HA antibody is useful for studies utilizing HA-tagged target proteins. HA Tag antibody produces CUT&RUN peaks (**Figure 2**) in breast cancer cells expressing 3xHA-tagged GATA3 transcription factor [1]*.

TECHNICAL INFORMATION

Immunogen	A synthetic HA peptide (sequence: YPYDVPDYA)
Storage	Stable for 1 year at 4°C from date of receipt
Formulation	Antigen affinity-purified antibody in phosphate buffered saline (PBS), 0.09% sodium azide

RECOMMENDED DILUTION

CUT&RUN	0.5 µg per reaction	**Western Blot**	1:1,000 - 1:30,000

REFERENCES

[1] Takaku et al. *Genome Biol.* (2016). PMID: 26922637

**Thanks to Dr. Takaku (UND) for 3xFlag-GATA3-3xHA MDA-MB-231 cells.*

VALIDATION DATA

CUT&RUN Methods

CUT&RUN was performed on 500k MDA-MB-231 nuclei stably expressing c-terminal 3xHA-tagged GATA3 [1]* with 0.5 µg of either HA Tag, H3K4me3 positive control (EpiCypher 13-0041), or IgG negative control (EpiCypher 13-0042) antibodies using the CUTANA™ ChIC/CUT&RUN Kit v2 (EpiCypher 14-1048). Library preparation was performed with 5 ng of 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 5.5 million reads (IgG), 9.9 million reads (H3K4me3), and 6.6 million reads (HA Tag). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

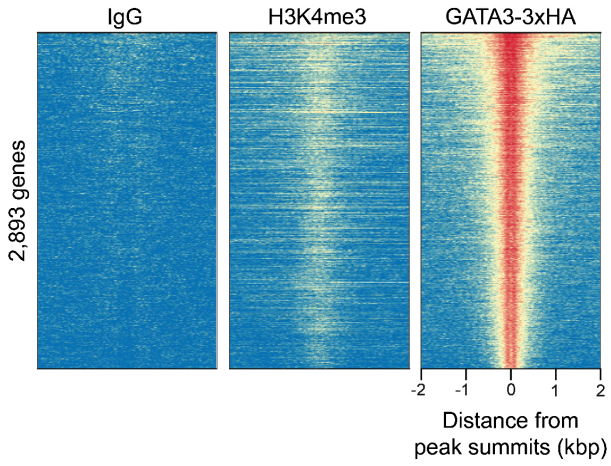

FIGURE 1 HA Tag peaks in CUT&RUN. CUT&RUN was performed as described above. Peaks were called using MACS2. Heatmaps show GATA3-3xHA peaks relative to IgG and H3K4me3 control antibodies in aligned rows ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal.

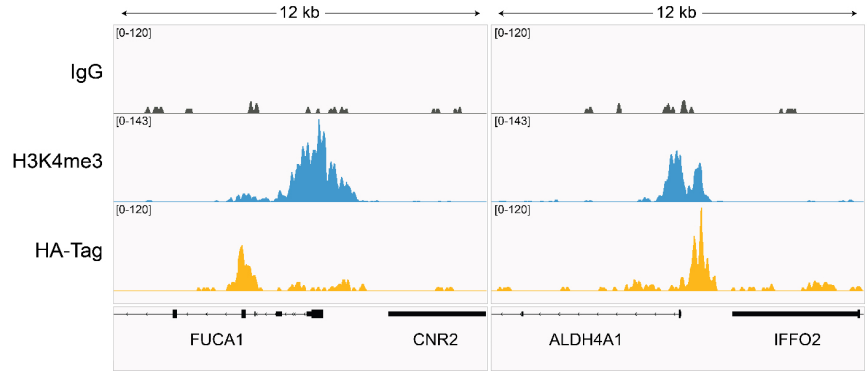

FIGURE 2 HA-tagged CUT&RUN representative browser tracks. CUT&RUN was performed as described above. Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). Two of the top called peaks are shown.

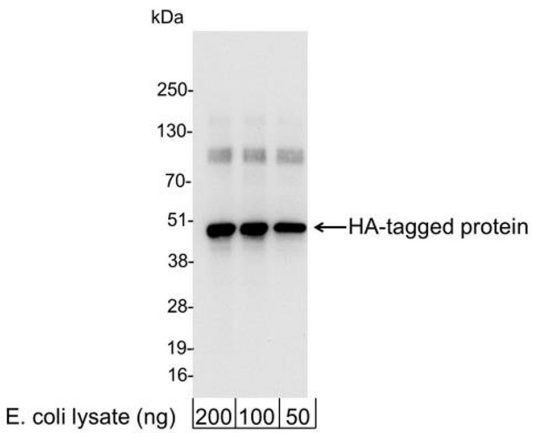

FIGURE 3 Western blot data. *E. coli* cells expressing a multi-tag fusion protein were used to prepare whole cell lysates. The indicated amounts (ng) of lysate were loaded onto a 4-20% SDS-PAGE gel and analyzed under standard western blot conditions using HA Tag antibody at a dilution of 1:25,000.

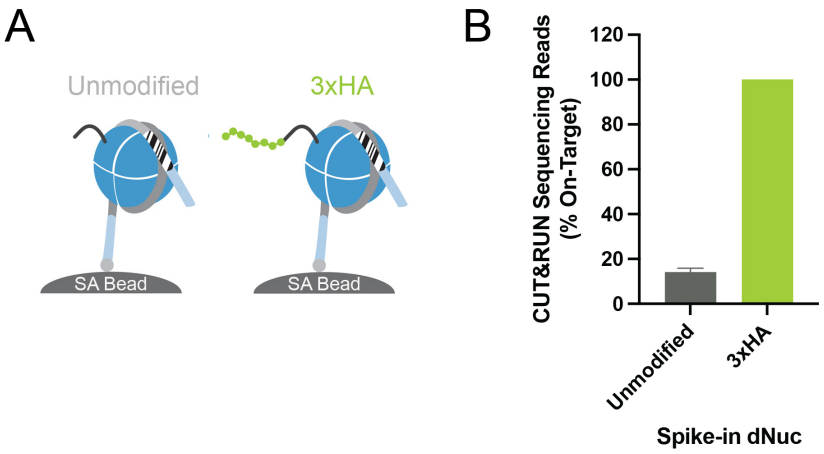

FIGURE 4 Target-specific epitope cleavage of HA Tag antibody in CUT&RUN was determined using DNA-barcoded recombinant nucleosome spike-in controls. (**A**) A recombinant nucleosome was created where epitope tag 3xHA was fused to the histone H3 tail. The fused nucleosome and an unmodified control were immobilized to streptavidin beads (SA Beads) and spiked into CUT&RUN samples alongside ConA bead immobilized MDA-MB -231 cells expressing GATA3-3xHA. HA Tag antibody and pAG-MNase (EpiCypher 15-1016) were then added to release antibody-bound nucleosomes into solution through pAG-MNase mediated cleavage of the linker DNA (light blue). This approach provided a defined experimental control to assess whether the HA Tag antibody selectively cleaved the target epitope with high specificity and minimal background. **(B)** CUT&RUN sequence reads were aligned to the unique DNA "barcodes" corresponding to each nucleosome in the spike-in panel. Data are expressed as the percent of reads recovered relative to the intended target (3xHA, set to 100%). This analysis confirms that the HA Tag antibody specifically liberated the target epitope-tagged nucleosome into solution.