HA Tag CUTANA™ CUT&RUN Antibody

Catalog No 13-2010
Lot No 21195001-02
Pack Size 100 µg

Type Polyclonal **Target Size** N/A **Reactivity** HA Epitope (YPYDVPDYA)
Host Rabbit **Format** Aff. Pur. IgG **Applications** CUT&RUN, WB

Product 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 CUTANA approach using EpiCypher optimized protocols (EpiCypher.com/resources/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 1**) that overlap with GATA3 DNA-binding motifs (**Figure 2**) in breast cancer cells expressing 3xHA-tagged GATA3 transcription factor [1]*.

Immunogen:

A synthetic HA peptide (sequence: YPYDVPDYA).

Formulation:

Antigen affinity-purified antibody (1.0 mg/mL) in phosphate buffered saline (PBS), 0.09% sodium azide.

Storage and Stability:

Stable for 1 year at 4°C from date of receipt.

Application Notes:

CUT&RUN: 0.5 µg
WB: 1:1,000 - 1:30,000

References:

[1] Takaku et al (2016) *Genome Biol.* 17:36.
**Thanks to Dr. Takaku (UND) for 3xFlag-GATA3-3xHA MDA-MB-231 cells.*

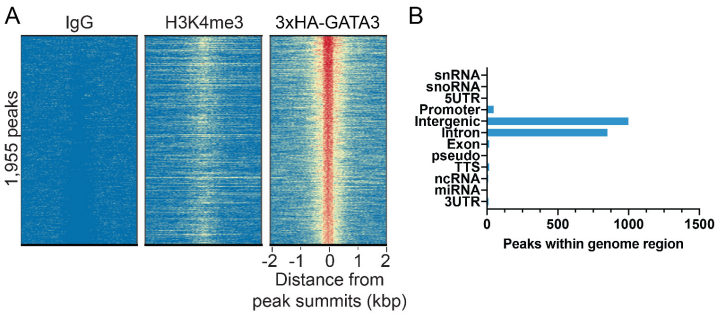

Figure 1: HA-tagged protein peaks in CUT&RUN. CUT&RUN was performed using HA antibody (0.5 µg) with 500,000 MDA-MB-231 cells stably expressing 3xHA-tagged GATA3 [1]*. Peaks were called using MACS2. (**A**) Heatmap showing 3xHA-GATA3 peaks relative to IgG and H3K4me3 control antibodies (EpiCypher 13-0042 and 13-0041, respectively) in aligned rows ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal. (**B**) The number of 3xHA-GATA3 peaks which fall into distinct classes of functionally annotated genomic regions is plotted.

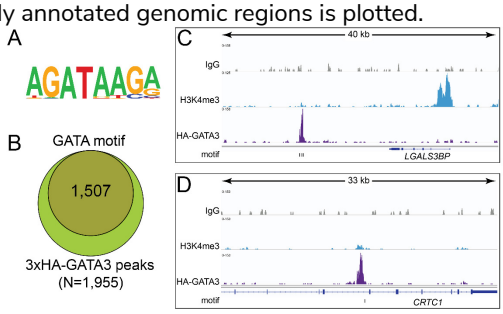

Figure 2: HA-tagged transcription factor binding motif analysis in CUT&RUN. (**A**) Homer analysis determined that the GATA3 consensus motif, represented as a sequence logo position weight matrix, was enriched under 3xHA-GATA3 CUT&RUN peaks. (**B**) The number of 3xHA-GATA3 peaks containing GATA3 consensus motifs from panel A is represented by a Venn Diagram. (**C-D**) Two representative loci show overlap of 3xHA-GATA3 peaks with the consensus motifs noted below (IGV).

Applications Key: ChIP: Chromatin immunoprecipitation; ChIP-seq: ChIP-sequencing; CUT&RUN: Cleavage Under Targets and Release Using Nuclease; CUT&Tag: Cleavage Under Targets and Tagmentation; E: ELISA; FACS: Flow cytometry; ICC: Immunocytochemistry; IF: Immunofluorescence; IHC: Immunohistochemistry; IP: Immunoprecipitation; L: Luminex; WB: Western Blot. **Reactivity Key**: B: Bovine; Ce: *C. elegans*; Ch: Chicken; Dm: *Drosophila*; Eu: Eukaryote; H: Human; M: Mouse; Ma: Mammal; R: Rat; Sc: *S. cerevisiae*; Sp: *S. pombe*; WR: Wide Range (predicted); X: Xenopus; Z: Zebrafish

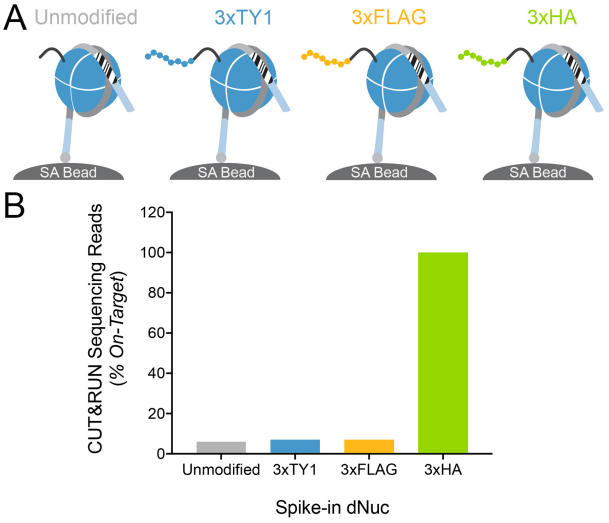

Figure 3: Target-specific epitope cleavage of HA Tag antibody in CUT&RUN was determined using DNA-barcoded recombinant nucleosome spike-in controls. (**A**) A panel of recombinant nucleosomes was created where various epitope tags (3xTY1, 3xFLAG, 3xHA) were fused to the histone H3 tail. The fused nucleosomes and an umodified control were immobilized to streptavidin beads (SA Bead) and spiked into CUT&RUN samples alongside ConA bead immobilized 3xHA MDA-MB -231 cells (Figure 1). 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.

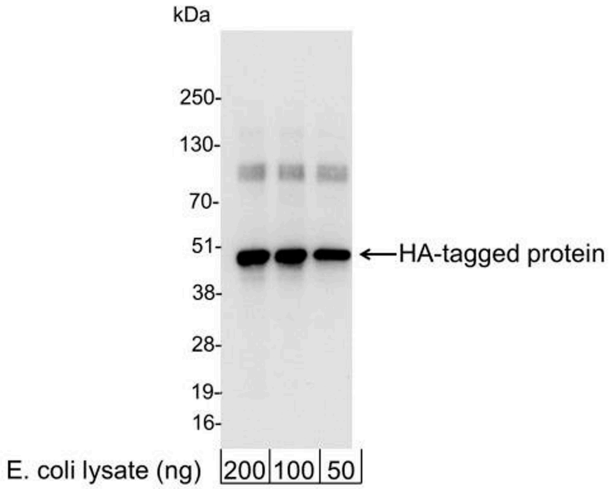

Figure 4: Western blot detection of HA-tagged protein. *E. coli* cells expressing a multi-tag fusion protein were used to prepare whole cell lysates. The indicated amounts (ng) of lysate were loaded onto 4-20% SDS-PAGE gel and analyzed under standard western blot conditions using HA Tag antibody (1:25,000).