DYKDDDDK Tag CUTANA™ CUT&RUN Antibody

Catalog No	13-2031	**Type**	Monoclonal
Lot No	24011001-81	**Host**	Rabbit
Pack Size	100 µL	**Concentration**	100 µg/mL
Applications	CUT&RUN, ELISA, WB, IP, ICC	**Reactivity**	FLAG® Epitope (DYKDDDDK)

DESCRIPTION

DYKDDDDK antibody is useful for studies utilizing FLAG®*-tagged target proteins. 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. DYKDDDDK Tag antibody produces CUT&RUN peaks (**Figures 2-3**) that overlap with GATA3 in breast cancer cells expressing 3xFLAG-tagged GATA3 transcription factor [1]**

**FLAG® is a registered trademark of Merck KGaA, Darmstadt, Germany.*
***Thanks to Dr. Takaku (UND) for 3xFLAG-GATA3-3xHA MDA-MB-231 cells.*

TECHNICAL INFORMATION

Immunogen	A synthetic peptide sequence (DYKDDDDK)
Storage	Stable for 1 year at 4°C from date of receipt
Formulation	Antigen affinity-purified recombinant monoclonal antibody in borate buffered saline (BBS) pH 8.2, 0.1% BSA, 0.09% sodium azide

RECOMMENDED DILUTION

CUT&RUN	0.05 µg per reaction (0.5 µL of 100 µg/mL antibody)
Enzyme-Linked Immunosorbent Assay	1:1,000 - 1:75,000; for coating plates 1:100 - 1:500
Western Blot	1:1,000
Immunoprecipitation	20 µL/mg lysate
Immunocytochemistry	1:1,000 - 1:5,000. Epitope retrieval with citrate buffer pH 6.0 is recommended for FFPE cell sections

REFERENCES

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

VALIDATION DATA

CUT&RUN Methods

CUT&RUN was performed on 500k MDA-MB-231 native cells either stably overexpressing 3xFLAG-tagged GATA3 [1] or containing vector control. Either DYKDDDDK Tag (0.05 µg), H3K4me3 (0.5 µg; EpiCypher 13-0041), GATA3 (0.5 µg; CST 5852), or IgG (0.5 µg, EpiCypher 13-0042) antibodies were used with the CUTANA™ ChIC/CUT&RUN Kit v3 (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). Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth (vector control cells/overexpressing cells) was 5.6/4.5 million reads (IgG), 7.8/6.7 million reads (H3K4me3), 5.8/4.7 million reads (GATA3), and 6.2/5.5 million reads (DYKDDDDK 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.

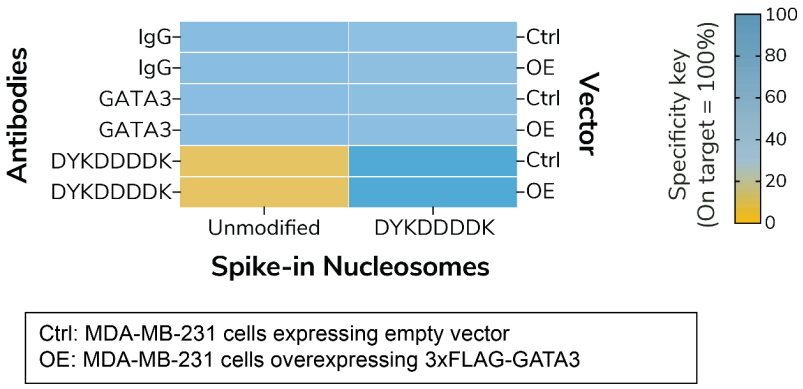

FIGURE 1 Defined nucleosome spike-ins provide an in-assay control for DYKDDDDK Tag antibody in CUT&RUN. CUT&RUN sequencing reads were aligned to the unique DNA barcodes corresponding to each nucleosome in the SNAP-CUTANA™ DYKDDDDK Tag Panel (EpiCypher 19-5001). Data are expressed as a percent relative to on-target recovery (DYKDDDDK Tag set to 100%) or total counts (IgG/GATA3). IgG/GATA3 show no preferential binding to unmodified or DYKDDDDK spike-in nucleosomes. DYKDDDDK Tag antibody selectively enriches the DYKDDDDK Tag spike-in nucleosome, validating the antibody in CUT&RUN.

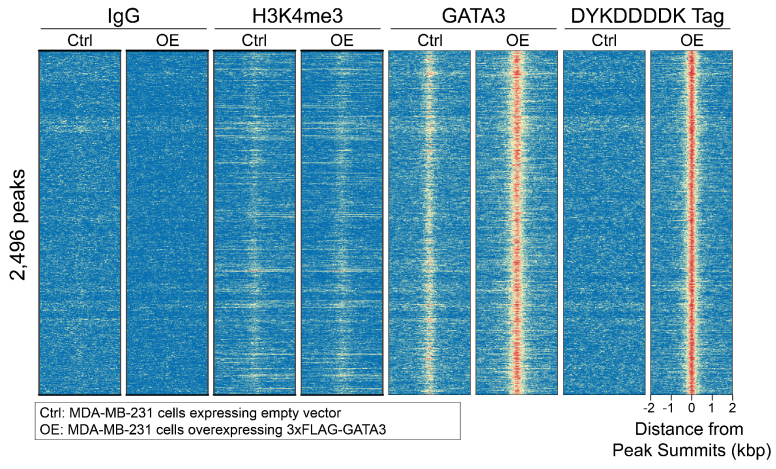

FIGURE 2 DYKDDDDK-tagged protein peaks in CUT&RUN. Heatmaps show DYKDDDDK Tag antibody-enriched peaks called for FLAG-GATA3 overexpressing cells (OE) in aligned rows relative to all other experimental conditions. Rows are ranked by intensity (top to bottom) and colored such that red indicates high localized enrichment and blue denotes background signal. A high degree of overlap is observed for DYKDDDDK and GATA3 antibodies as expected in the OE cells, while empty vector control (Ctrl) shows absence of FLAG and lower GATA3 enrichment representing endogenous protein. IgG shows low background signal. H3K4me3, a canonical mark of promoters, does not appear in regions of high GATA3 enrichment.

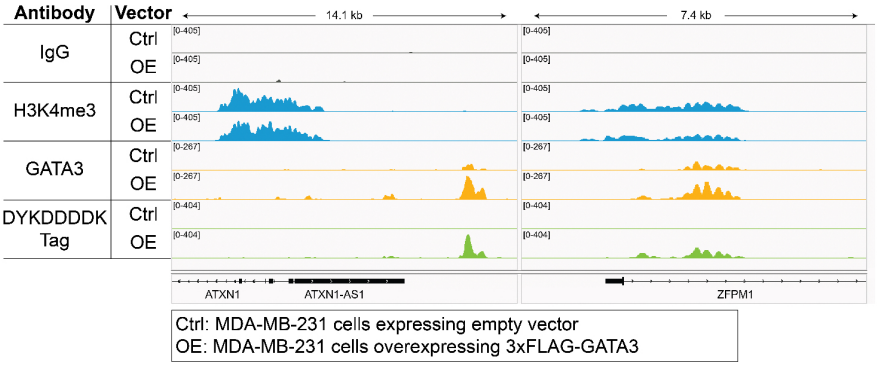

FIGURE 3 CUT&RUN representative browser tracks for DYKDDDDK-tagged protein. Gene browser shots were generated using the Integrative Genomics Viewer (IGV, Broad Institute). Two of the top called peaks in FLAG-GATA3 overexpressing (OE) cells are shown. The peaks show the same distribution patterns as observed in the genome-wide heatmaps (**Figure 2**). IgG shows low background, H3K4me3 is unchanged between empty vector control (Ctrl) and OE cells, GATA3 peaks are more robust in OE cells, and peaks overlap between GATA3 and FLAG antibodies in OE cells. These results demonstrate the robustness and specificity of DYKDDDDK tag antibody in CUT&RUN experiments targeting FLAG-tagged proteins.

VALIDATION DATA

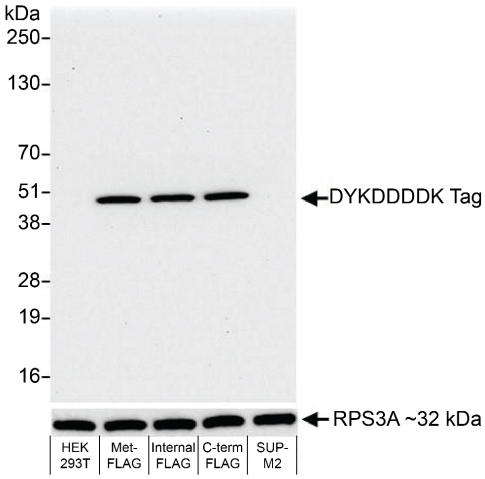

FIGURE 4 Western blot data. Western analysis of FLAG-tagged protein in lysates from non-transfected human HEK293 cells, HEK293 transfected with Met-FLAG-IDO1, HEK293 transfected with IDO1-FLAG-HIS (Internal Tag), HEK293 transfected with IDO1-HIS-FLAG (C-Terminal Tag), and SUP-M2 cells. EpiCypher DYKDDDDK Tag antibody was used at 1:1,000. An HRP-conjugated goat anti-rabbit IgG antibody (Fortis A120-101P) was used as a secondary antibody. Chemiluminescence exposure time was 10 seconds. Lower panel shows rabbit anti-RPS3A antibody results (Fortis A305-003A).

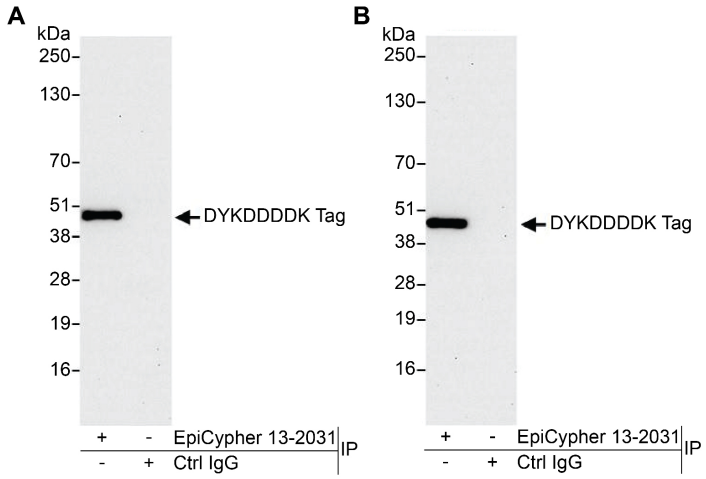

FIGURE 5 Immunoprecipiation data. EpiCypher DYKDDDDK Tag antibody (20 µL/mg lysate) was used to immunoprecipitate lysates isolated from HEK293 cells transfected with either Met-FLAG-IDO1 (**A**) or IDO1-HIS-FLAG (C-terminal tag; **B**). For blotting immunoprecipitated DYKDDDDK Tag, EpiCypher DYKDDDDK Tag antibody was used at 1:1,000. Chemiluminescence exposure time was 10 seconds.

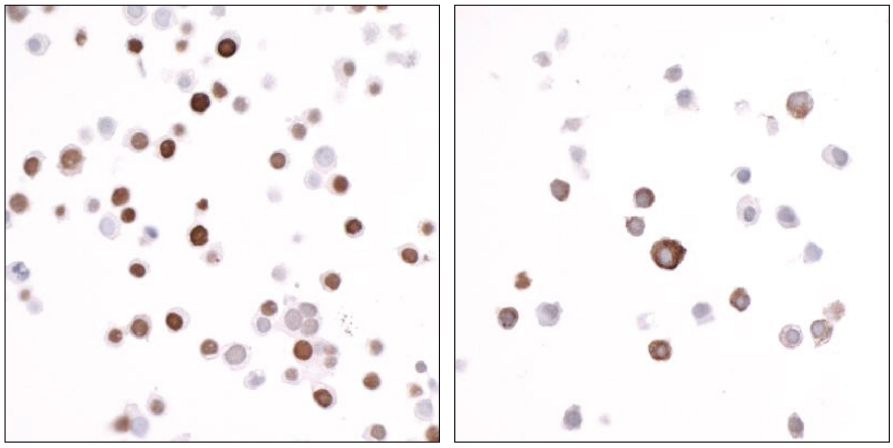

FIGURE 6 Immunocytochemistry data. FFPE sections of HEK293 cells expressing either FLAG-tagged nuclear protein (**left**) or FLAG-tagged cytoplasmic protein (**right**) using EpiCypher DYKDDDDK Tag antibody. An HRP-conjugated goat anti-rabbit IgG antibody (Fortis A120-501P) was used as a secondary antibody. The substrate used was DAB.