CUTANA™ pAG-Tn5 for CUT&Tag

Catalog No	15-1017	**Species**	*E. coli*
Lot No	23243004-C1	**Source**	*E. coli*
Pack Size	50 Reactions	**Epitope Tag**	None
Concentration	20X	**MW**	191 kDa

DESCRIPTION

CUTANA pAG-Tn5 is the key reagent for performing efficient mapping of chromatin features in Cleavage Under Targets and Tagmentation (CUT&Tag) [1]. CUT&Tag offers significant improvements in signal to noise at reduced cell inputs and sequencing depth compared to ChIP-seq. As a fusion of Proteins A and G to a highly active *E. coli* transposase mutant (Tn5), CUTANA pAG-Tn5 is compatible with target antibodies from a broad spectrum of host species. It also comes without an epitope tag, making it suitable for tag-mediated CUT&Tag (e.g. FLAG, HA, TY1, V5, etc.). This product is highly purified to remove contaminating *E. coli* DNA, which can be tagmented during storage and complicate analysis at low cell numbers. For superior normalization as well as antibody validation and reaction monitoring, SNAP-CUTANA™ nucleosome spike-ins (e.g. EpiCypher 19-1002) are recommended. The Tn5 comes charged with mosaic adapters and is ready to be used in CUT&Tag.

RECOMMENDED ACCESSORY REAGENTS

Item	CAT	Item	CAT
Anti-Rabbit Secondary Antibody	13-0047	H3K27me3 Positive Ctrl Antibody	13-0055
Anti-Mouse Secondary Antibody	13-0048	Rabbit IgG Negative Ctrl Antibody	13-0042
Magnetic Separation Rack, 0.2 mL	10-0008	CUTANA™ ConA Beads	21-1401
Magnetic Separation Rack, 1.5 mL	10-0012	SNAP-CUTANA™ K-MetStat	19-1002
CUT&RUN 8-strip Tubes	10-0009	Non-HS 2X PCR Master Mix	15-1018

TECHNICAL INFORMATION

Storage Stable for one year at -20°C from date of receipt. The protein is not subject to freeze/thaw under these conditions.

Formulation 50 mM HEPES-KOH pH 7.2, 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 50% glycerol

Adapters Tn5ME-A: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'
Tn5ME-B: 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'
Tn5ME-rev: 5'-[phos]CTGTCTCTTATACACATCT-3'

APPLICATION NOTES

Add 2.5 µL of the supplied enzyme to a 50 µL CUT&Tag reaction (20X dilution). For detailed applications and uses of this product, please see epicypher.com/protocols.

REFERENCES

[1] Kaya-Okur et al. *Nat. Commun.* (2019). PMID: 31036827

VALIDATION DATA

CUT&Tag Methods

CUT&Tag was performed on 100k K562 cells with 0.5 µg of either IgG (EpiCypher 13-0042), H3K4me3 (EpiCypher 13-0041), or H3K27me3 (EpiCypher 13-0055) antibodies using CUTANA™ pAG-Tn5 (1:20 dilution) and the CUTANA™ CUT&Tag Kit v1 (EpiCypher 14-1102/14-1103. Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 11.6 million reads (IgG), 12.8 million reads (H3K4me3), and 17.7 million reads (H3K27me3). Data were aligned to the hg19 genome using Bowtie2. Data were filtered to remove duplicates, multi-aligned reads, and ENCODE DAC Exclusion List regions.

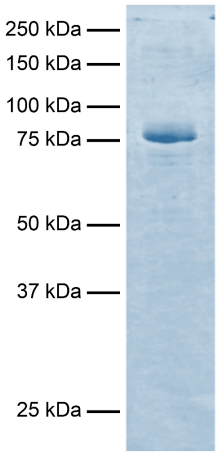

FIGURE 1 Protein gel data. CUTANA pAG-Tn5 for CUT&Tag (1 µg) was resolved via SDS-PAGE and stained with Coomassie blue. The migration and molecular weight of the protein standards are indicated. Uncharged pAG-Tn5 monomer is 78.5 kDa, however once charged with DNA, Tn5 dimerizes to a final complex weight of 191 kDa.

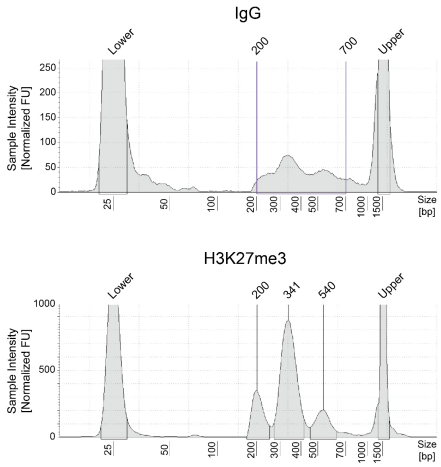

FIGURE 2 Size distribution of released chromatin. CUT&Tag was performed as described above. Recovered DNA was directly PCR amplified to produce sequence-ready libraries. Agilent TapeStation traces for libraries derived from negative control IgG (top) and H3K27me3 (bottom) antibodies are shown. Excised DNA is highly enriched for mononucleosomes (peak at ~300 bp reflects ~150 bp insert size).

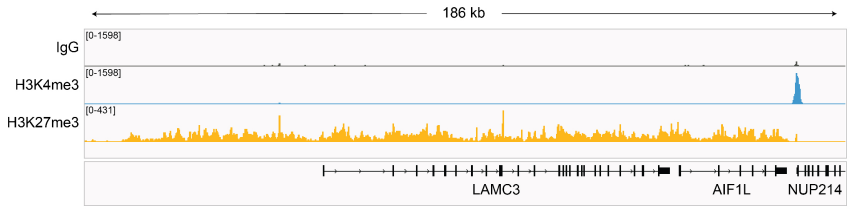

FIGURE 3 CUT&Tag data. CUT&Tag was performed as described above. Representative sequencing tracks obtained using CUTANA pAG-Tn5 show a 186 kb close up view of the LAMC3 gene. CUTANA pAG-Tn5 produced clear peaks with genomic distribution profiles consistent with the known biological functions of H3K4me3 and H3K27me3 as well as minimal background in the IgG negative control.

US Pat. No. 10,689,643, 11,306,307, EU Pat. No. 3,688,157, 2,999,784
and related patents and pending applications