CUTANA™ Uncharged 6xHis-pAG-Tn5 for CUT&Tag

Catalog No	15-1028	**Species**	*E. coli*
Lot No	24092001-01	**Source**	*E. coli*
Pack Size	75 µL	**Epitope Tag**	6xHis
Concentration	1.5 µM (dimer)	**MW**	161,401.8 Da (dimer)

DESCRIPTION

Products in EpiCypher's IDEA Toolbox (Innovation and Discovery of Epigenetic Applications) offer access to reagents without known or fully defined uses, enabling researchers to explore cutting-edge applications. Due to their novelty and unexplored potential, EpiCypher will engage in limited technical support.

CUTANA™ Uncharged 6xHis-pAG-Tn5 is a fusion of proteins A and G to *E.coli* transposase (Tn5), the key enzyme for CUT&Tag [1]. The fusion protein contains a polyhistidine tag (6xHis) to enable applications that require affinity purification steps (for an untagged version of uncharged pAG-Tn5, see EpiCypher 15-1025). **Uncharged Tn5 must be loaded with user-designed mosaic adapter DNA prior to use in CUT&Tag** (see reference [1] and Application Notes). His-pAG-Tn5 enables purification of differentially barcoded antibody-Tn5 complexes such as those described in MulTI-Tag (Multiple Target Identification by Tagmentation) and multi-CUT&Tag workflows to simultaneously interrogate multiple chromatin proteins in a single CUT&Tag reaction [2,3]. These approaches have been applied to map histone post-translational modifications and RNA Polymerase II down to single cell resolution to define cell-specific epigenetic landscapes.

RECOMMENDED ACCESSORY PRODUCTS

Item	CAT	Item	CAT
CUTANA™ 8-strip Tubes	10-0009	H3K4me3 Positive Control Antibody	13-0060
Magnetic Separation Rack, 0.2/1.5 mL	10-0008/10-0012	H3K27me3 Positive Control Antibody	13-0055
Nuclei Extraction Buffer	21-1026	Anti-Rabbit Secondary Antibody	13-0047/13-1047
CUTANA™ ConA Beads	21-1401/21-1411	Anti-Mouse Secondary Antibody	13-0048/13-1048
SNAP-CUTANA™ K-MetStat	19-1002	Non-HS 2X PCR Master Mix	15-1018
Rabbit IgG Negative Control Antibody	13-0042	Quick Cleanup DNA Purification Kit	14-0052

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.

APPLICATION NOTES

pAG-Tn5 transposomes can be assembled as previously described [1]. In brief, 3.2 µL of an equimolar mixture of pre-annealed user-defined Adapter-A and user-defined Adapter-B oligonucleotides (50 µM each, 100 µM total adapter DNA) should be mixed with 70 µL of 1.5 µM pAG-Tn5 fusion protein dimer (a 3:1 molar ratio of adapter DNA to pAG-Tn5 dimer). The mixture is then incubated on a rotating platform for 1 hour at room temperature and stored at -20°C. Specific activity definition of the charged pAG-Tn5 is highly recommended before use in CUT&Tag. **Due to the confounding variable of user-supplied mosaic adapters, EpiCypher will not engage in protocol troubleshooting for this reagent.**

REFERENCES

[1] Kaya-Okur et al. *Nat. Commun.* (2019). PMID: 31036827
[2] Meers et al. *Nat. Biotechnol.* (2023). PMID: 36316484
[3] *Gopalan et al. Mol. Cell (2021). PMID: 34637755*

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VALIDATION DATA

CUT&Tag Methods

6xHis-pAG-Tn5 was charged with EpiCypher's standard adapters (see "Technical Information" for EpiCypher 15-1017) using the loading protocol described in Application Notes above. CUT&Tag was performed on 100k native K562 nuclei with 0.5 µg of either IgG (EpiCypher 13-0042), H3K4me3 (EpiCypher 13-0041), or H3K27me3 (EpiCypher 13-0055) antibodies using CUTANA™ Uncharged 6xHis-pAG-Tn5 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 4.8 million reads (IgG), 9.0 million reads (H3K4me3), and 5.4 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.

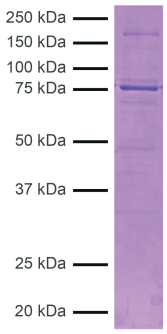

FIGURE 1 Protein gel data. CUTANA Uncharged 6xHis-pAG-Tn5 (1 µg) was resolved via SDS-PAGE and stained with Coomassie blue. The migration and molecular weight of the protein standards are indicated.

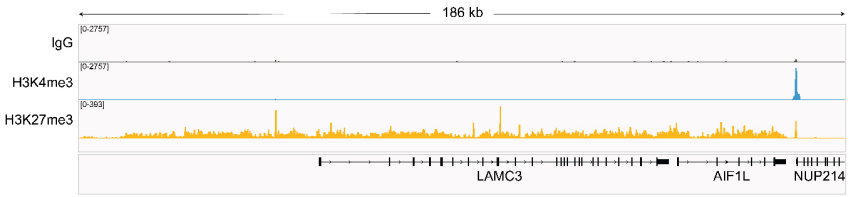

FIGURE 2 Functional validation in CUT&Tag. CUT&Tag was performed as described above. Representative sequencing tracks obtained using CUTANA Uncharged 6xHis-pAG-Tn5 show a 186 kb close up view of the LAMC3 gene. CUTANA Uncharged 6xHis-pAG-Tn5 produced clear peaks with genomic distribution profiles consistent with the known biological functions of H3K4me3 and H3K27me3 as well as minimal background in the IgG negative control.

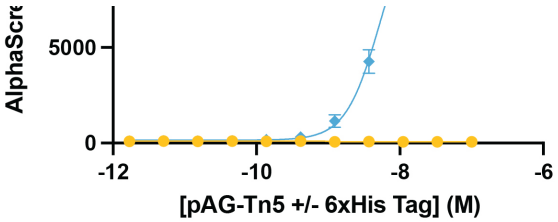

FIGURE 3 Functional validation of 6xHis binding. Various concentrations of CUTANA Uncharged 6xHis-pAG-Tn5 were incubated with biotinylated recombinant nucleosomes (rNuc; EpiCypher 16-0006). Uncharged pAG-Tn5 lacking a 6xHis tag (EpiCypher 15-1025) was used as a negative control. AlphaScreen technology (PerkinElmer/Revvity) was used to confirm functional 6xHis binding by using Nickel Chelate Donor Beads (Revvity AS101) to bind the 6xHis tag and Streptavidin Acceptor Beads (Revvity AL125) to bind biotin-rNuc. Signal (AlphaScreen counts) indicates 6xHis-pAG-Tn5 complexed with biotin-rNuc. No signal was observed in pAG-Tn5 lacking a 6xHis tag.

US Pat. No. 10689643, 11306307, 11733248; EU Pat. No. 3688157, 2999784
and related patents and pending applications

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