CUTANA™ Fluorescent pAG-Tn5 for CUT&Tag

Catalog No	15-1026	**Species**	*E. coli*
Lot No	22200001-21	**Source**	*E. coli*
Pack Size	125 µL	**Epitope Tag**	None
Concentration	1 µM	**MW**	192 kDa

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 Fluorescent pAG-Tn5 is a fusion of Proteins A and G to *E. coli* transposase (Tn5), the key enzyme for CUT&Tag [1]. The Mosaic A and Mosaic B adapters loaded in this enzyme bear a 5' Cy5 fluorescent dye to enable multiomic visualization and mapping of chromatin features. This product is highly purified to remove contaminating *E. coli* DNA. For an enzyme charged with non-fluorescent adapters that can be used in standard CUT&Tag, see EpiCypher 15-1017.

RECOMMENDED ACCESSORY REAGENTS

Item	CAT	Item	CAT
Anti-Rabbit Secondary Antibody	13-0047	H3K4me3 Positive Ctrl Antibody	13-0041
Anti-Mouse Secondary Antibody	13-0048	Rabbit IgG Negative Ctrl Antibody	13-0042
Non-Hot Start 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'-[Cy5]TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'
Tn5ME-B: 5'-[Cy5]GATTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'
Tn5ME-rev: 5'-[phos]CTGTCTCTTATACACATCT-3'

APPLICATION NOTES

A 20X dilution of this reagent is sufficient for a standard CUT&Tag reaction following the EpiCypher CUTANA™ Direct-to-PCR CUT&Tag protocol (epicypher.com/protocols), indicating that addition of the fluorescent dye does not prevent Tn5-mediated tagmentation of chromatin (see Figures 2 & 3). However, application-specific optimization will be necessary for use in microscopy. Due to the novelty of this reagent, EpiCypher will not engage in application tech support.

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 (ThermoFisher MA5-11198) antibodies using CUTANA™ Fluorescent pAG-Tn5 (50 nM final) following the EpiCypher Direct-to-PCR CUT&Tag protocol (epicypher.com/protocols). Libraries were run on an Illumina NextSeq2000 with paired-end sequencing (2x50 bp). Sample sequencing depth was 0.5 million reads (IgG), 2.5 million reads (H3K4me3), and 6.3 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.

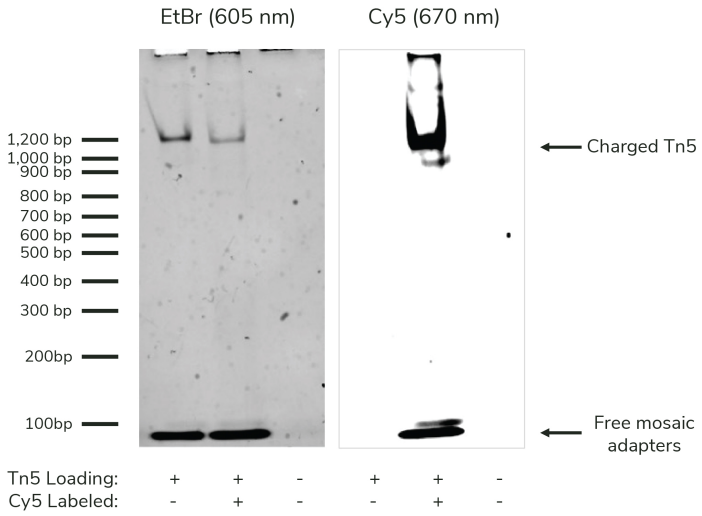

FIGURE 1: DNA charging data. CUTANA Fluorescent pAG-Tn5 for CUT&Tag (0.5 µg) was resolved via Native PAGE alongside uncharged pAG-Tn5 (EpiCypher 15-1025) and charged pAG-Tn5 (EpiCypher 15-1017). The gel was imaged and Cy5 emission was observed at 670 nm (right), stained with ethidium bromide, and imaged again at 605 nm. The observed shift indicates successful loading of the Tn5, and fluorescent imaging confirms the presence of the dye.

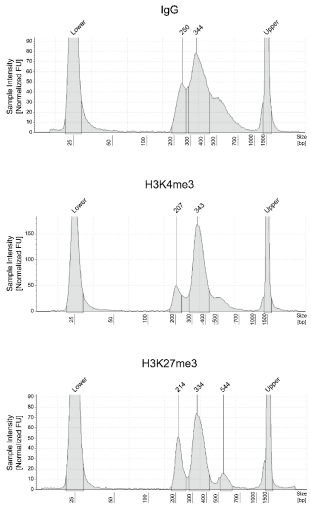

FIGURE 2: Size distribution of released chromatin. CUT&Tag was performed as described above. Recovered DNA was directly PCR amplified to produce sequence-ready libraries. Agilent TapeStation traces for libraries derived from negative control Rabbit IgG (top), H3K4me3 (middle), and H3K27me3 (bottom) are shown. Excised DNA is highly enriched for mononucleosomes (peak at ~300 bp reflects ~150 bp insert size)

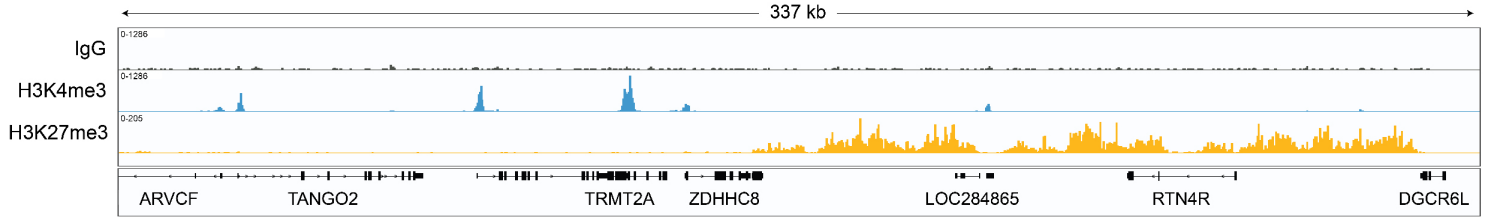

FIGURE 3: CUT&Tag data. CUT&Tag was performed as described above. Representative sequencing tracks obtained using CUTANA Fluorescent pAG-Tn5 for CUT&Tag show a 337 kb view of the TRMT2A gene. CUTANA pAG-Tn5 produced clear peaks with genomic distribution consistent with the known biological functions of H3K4me3 and H3K27me3 as well as minimal background in the IgG negative control.

VALIDATION DATA

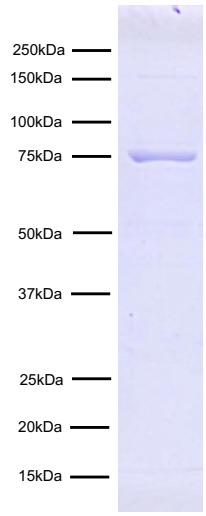

FIGURE 4: Protein gel data. CUTANA pAG-Tn5 (1 µg) was resolved via SDS-PAGE and stained with Coomassie blue. The migration and molecular weight of the protein standards are indicated. Uncharged pAG-Tn5 monomer is 78.5 kDa, however once charged with DNA, Tn5 dimerizes to a final complex weight of 192 kDa.