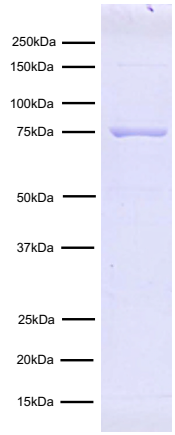
CUTANA™ pAG-Tn5 for CUT&Tag

Catalog No. 15-1017
Lot No. 20142001-C1
Pack Size 50 reactions

Type	Transposase	**Expressed In**	*E. coli*
Mol. Wgt.	191 kDa	**Epitope Tag**	None

Product Description:

Recombinantly produced in *E. coli*, CUTANA pAG-Tn5 for CUT&Tag is a fusion of Proteins A and G to Transposase Tn5. This construct is useful in performing Cleavage Under Targets and Tagmentation (CUT&Tag). The active dimer of Transposase Tn5 is charged with Illumina adapters and ready to be used immediately in CUT&Tag. The fusion does not contain an epitope tag, which makes it compatible with CUT&Tag for epitope tagged proteins (FLAG, HA, TY1, etc.).



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.

Formulation:

CUTANA pAG-Tn5 is provided as a 20X stock in storage buffer (50 mM HEPES-KOH pH 7.2, 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 50% glycerol).

Storage and Stability:

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

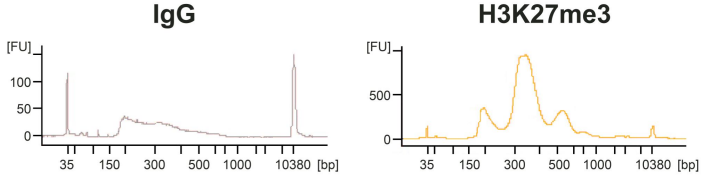
Application Notes:

This product is sufficient to perform 50 CUT&Tag reactions.

Recommended use: 2.5 µL of the supplied enzyme into a 50 µL CUT&Tag reaction (20X dilution). For detailed applications and uses of this product, please see CUT&Tag protocol at: epicypher.com/resources/protocols

Adapter Sequences:

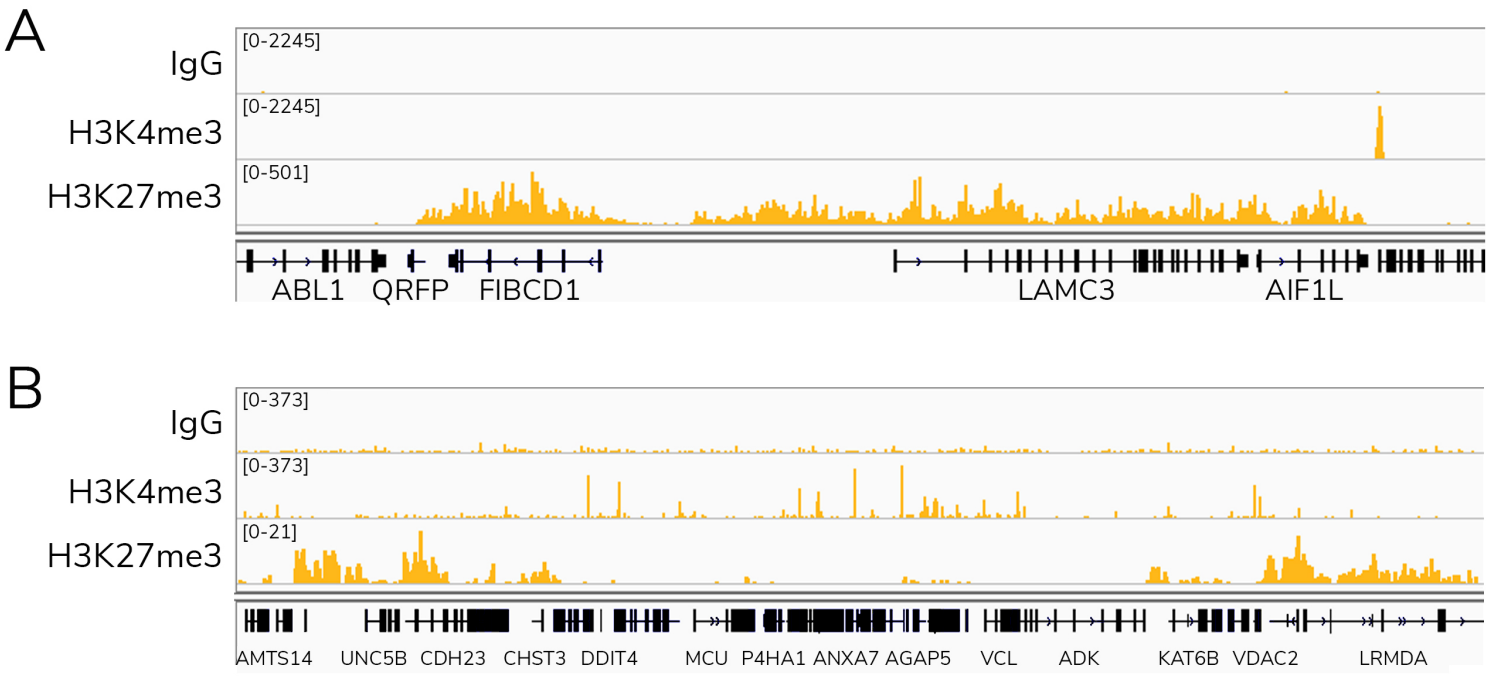
Tn5ME-A: 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'
Tn5ME-B:5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'
Tn5MErev: 5'-[phos]CTGTCTCTTATACACATCT-3'



Size Distribution of Released Chromatin: CUT&Tag was performed using CUTANA pAG-Tn5 (1:20 dilution) with 100,000 K-562 nuclei. Recovered DNA was directly PCR amplified to produce sequence-ready libraries. Agilent Bioanalyzer traces for libraries derived from negative control Rabbit IgG (left; EpiCypher Catalog No. 13-0042, Lot 20036001-52) and H3K27me3 (right; EpiCypher Catalog No. 13-0030, Lot 18303001) are shown. Excised DNA is highly enriched for mononucleosomes (peak at ~300 bp reflects 150 bp insert size).

References:

(1) Kaya-Okur et. al, *Nat. Commun.* 2019 (PMID : 31036827)
(2) Henikoff and Henikoff, *bioRxiv*. 2020 (2020.04.15.043083)



CUT&Tag Data: Representative sequencing tracks obtained using CUTANA pAG-Tn5 are shown for two different loci: a 300 kb close up view of the LAMC3 gene (**A**) and a wide 5,608 kb window (**B**). CUT&Tag was performed using 100,000 K-562 nuclei with H3K4me3 antibody (EpiCypher Catalog No. 13-0041, Lot 20083002-42), H3K27me3 antibody (EpiCypher Catalog No. 13-0030, Lot 18303001) and negative control Rabbit IgG (EpiCypher Catalog No. 13-0042, Lot 20036001-52). Total paired-end read counts were 0.7, 1.1, and 0.9 million reads for H3K4me3, H3K27me3, and IgG, respectively. Images were generated using the Integrative Genomics Viewer (IGV, Broad Institute). CUTANA pAG-Tn5 produced clear peaks with genomic distribution profiles consistent with the known biological functions of H3K4me3 and H3K27me3.

Application Notes Addendum: Since CUT&Tag has lower background and is compatible with fewer cells compared to ChIP-seq, it is not recommended to assess fragment size distribution using agarose gel or capillary electrophoresis (e.g. Agilent Bioanalyzer or TapeStation) prior to library amplification. This analysis is not indicative of the success of a CUT&Tag experiment, and further the amount of DNA recovered may be below the sensitivity of detection for these approaches. To gauge the success of a CUT&Tag experiment, assess DNA yield compared to positive (e.g. H3K4me3, H3K27me3) and negative (IgG) controls, determine fragment size distribution of sequence-ready libraries, and evaluate peak structure and expected genome-wide distribution in NGS data.