Store at -20°C

CUT&Tag pAG-Tn5 (Loaded)

#79561

50 assays

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Description: The CUT&Tag pAG-Tn5 (Loaded) enzyme provides enough enzyme to support 50 CUT&Tag assays. It is a fusion of Protein A and Protein G to Tn5, and is recombinantly produced in E. coli. This pAG-Tn5 has been loaded with the adaptor oligo that is compatible with NG-Sequencing for Illumina systems, so the genomic DNA fragments tagmented by CUT&Tag pAG-Tn5 (Loaded) are ready for PCR amplification and NG-seq. This enyzme is compatible with multiple species of antibodies, including both rabbit and mouse. This enzyme is validated using CUT&Tag Assay Kit #77552.

Background: Similar to Cleavage Under Targets and Release Using Nuclease (CUT&RUN), Cleavage Under Targets and Tagmentation (CUT&Tag) is a powerful technique used for probing protein-DNA interactions within the natural chromatin context of the cell (1-3). CUT&Tag has many of the same advantages as the CUT&RUN assay in that it provides a rapid, robust, and true low cell number protocol for detection of protein-DNA interactions in the cell. In addition, the CUT&Tag assay adds an in situ adaptor DNA ligation step carried out by the pAG-Tn5 enzyme, in which an adaptor DNA is ligated directly to antibody-targeted chromatin DNA fragments in the cell. As a result, subsequent DNA library preparation is much faster and easier than library preparation following the CUT&RUN assay, free from DNA end repair, A-tailing, and adaptor ligation *in vitro*. CUT&Tag works very well for analyzing histone modifications, in addition to mapping some transcription factor and cofactor binding.

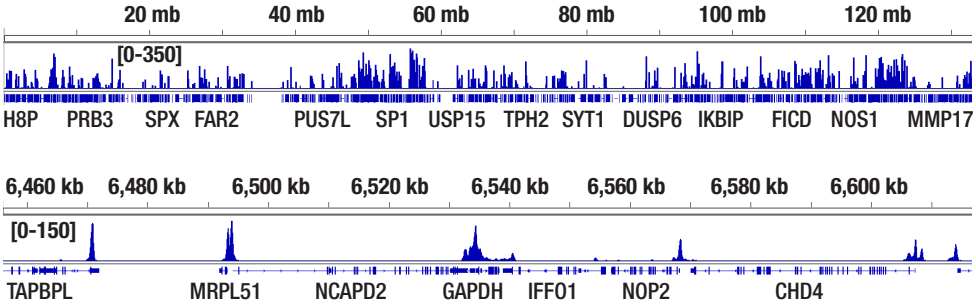

Figure 1. CUT&Tag was performed with HCT 116 cells and Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751, using CUT&Tag pAG-Tn5 (Loaded) and CUT&Tag Assay Kit #77552. The DNA library was prepared using CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems #47415. The figure shows binding across chromosome 12 (upper), including GAPDH (lower), a known target gene of H3K4me3.

Storage: Supplied in 22 mM HEPES pH 7.4, 44 mM NaCl, 44 µM EDTA, 0.4 mM DTT, 0.04% Triton X-100, and 50% glycerol. Store at –20°C and do not aliquot. This product is stable for 6 months.

Directions for Use: Please refer to CUT&Tag Assay Kit #77552 for detailed use of this enzyme in the CUT&Tag assay. After cell permeabilization and primary and secondary antibody binding, resuspend cells in 50 µL of High Salt Digitonin Buffer containing 2 µL of pAG-Tn5 Enzyme (1:25 dilution). Incubate cell samples at room temperature for 1 hour, wash cells with High Salt Digitonin Buffer, and then perform the chromatin tagmentation.

Background References:

(1) Kaya-Okur, H.S. et al. (2019) *Nat Commun* 10, 1930.
(2) Kaya-Okur, H.S. et al. (2020) *Nat Protoc* 15, 3264-3283.
(3) Henikoff, S. et al. (2021) *Bio Protoc* 11, e4043.

Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
Species Cross-Reactivity: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse
All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

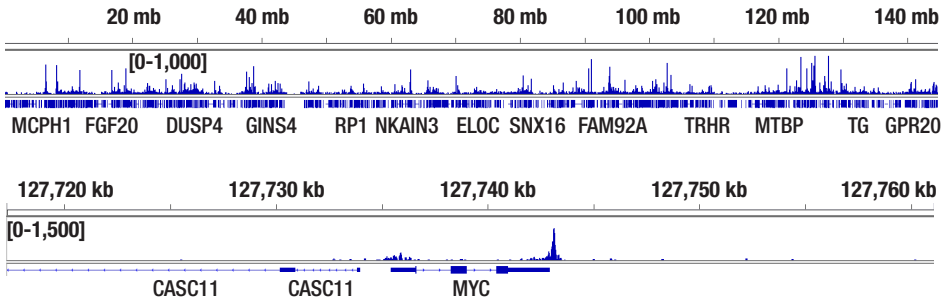

Figure 2. CUT&Tag was performed with HCT 116 cells and TCF4/TCF7L2 (C48H11) Rabbit mAb #2569, using CUT&Tag pAG-Tn5 (Loaded) and CUT&Tag Assay Kit #77552. The DNA library was prepared using CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems #47415. The figure shows binding across chromosome 8 (upper), including MYC (lower), a known target gene of TCF4.

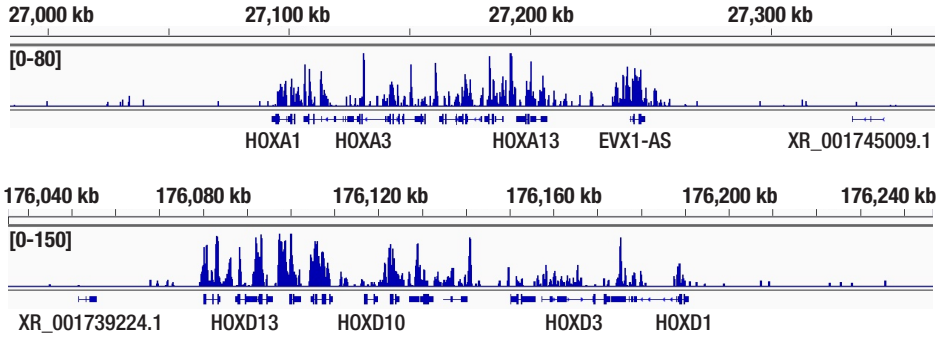

Figure 3. CUT&Tag was performed with NCCIT cells and JARID2 (D6M9X) Rabbit mAb #13594, using CUT&Tag pAG-Tn5 (Loaded) and CUT&Tag Assay Kit #77552. The DNA library was prepared using CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems #47415. The figure shows binding across HOXA (upper) and HOXD (lower), known target genes of JARID2.