Store at -20°C

CUT&Tag PCR Master Mix

#63228

840 µL

Cell Signaling TECHNOLOGY®

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Description: The CUT&Tag PCR Master Mix is an optimized 2X reaction mix for PCR amplification and DNA library preparation for CUT&Tag DNA samples. The non hot-start DNA polymerase in this product ensures the success of gap filling extension for tagmentated DNA. This master mix formulation is supplied at 2X concentration and contains all PCR components required for amplification of DNA, except primers and a DNA template.

This product is provided in 840 µL volumes sufficient for preparation of 24 PCR reactions, and is compatible with CUT&Tag DNA sample generated by CUT&Tag Assay Kit #77552 or CUT&Tag pAG-Tn5 (Loaded) #79561 and the index primers provided in the CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems #47415. This product is not compatible with library preparation for DNA samples from SimpleChIP® Chromatin IP Kits (#9003, #9005, #56383) or the CUT&RUN Assay Kit #86652.

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.

Storage: Store at -20°C. This product is stable for 18 months if stored properly.

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.

#63228 CUT&Tag PCR Master Mix Protocol

CUT&Tag PCR Master Mix

The CUT&Tag PCR Master Mix is an optimized 2X reaction mix for PCR amplification and DNA library preparation for CUT&Tag DNA samples. The non-hot start DNA polymerase in this product ensures the success of gap filling extension for tagmentated DNA. This master mix formulation is supplied at 2X concentration and contains all PCR reaction components required for amplification and quantitation of DNA, except primers and the tagmented DNA sample. This product is provided in 840 µL volumes sufficient for preparation of 24 PCR reactions and is compatible with CUT&Tag DNA samples generated by CUT&Tag pAG-Tn5 (Loaded) #79561 or DNA samples from other tagmentation assays, such as ATAC-seq. This product is recommended to be used with the index primers provided in the CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems #47415. Alternatively, other tagmentation PCR amplification indexes designed for downstream sequencing with Illumina system can be used. This product should not be used for library preparation of DNA samples from ChIP or CUT&RUN assays.

Compatible Reagents:

1. CUT&Tag pAG-Tn5 (Loaded) #79561
2. CUT&Tag Dual Index Primers and PCR Master Mix for Illumina Systems #47415

Non-Compatible Assay kit:

1. DNA Library Prep Kit for Illumina Systems (ChIP-seq, CUT&RUN) #56795

Required Reagents

Reagents Included:

a. CUT&Tag PCR Master Mix #63228

Reagents Not Included:

1. Forward and Reverse PCR indexes for tagmentated DNA
2. PCR tubes or plate and PCR Machine
3. AMPure XP Beads (Beckman Coulter, Inc. #A63881) or SPRIselect Reagent Kit (Beckman Coulter, Inc. #B23317)
4. 80% Ethanol (freshly prepared)
5. 10 mM Tris-HCl (pH 8.0-8.5)
6. Magnetic rack/stand
7. Agilent Bioanalyzer system and Agilent High Sensitivity DNA Kit (5067-4626)

CUT&Tag PCR Master Mix Protocol

SAFE STOP This is a safe stopping point in the protocol, if stopping is necessary.

I. Set up the PCR Reaction

Before starting:

- Thaw forward and reverse PCR indexes and CUT&Tag DNA (or any tagmentated DNA) at room temperature. Quick spin to collect all liquid from the sides of the tube.

1. Add the following components to a sterile PCR tube or single well of a PCR plate. Record the CUT&Tag Index 5 and CUT&Tag Index 7 added to each PCR tube or well.

Reagents	Volume for 1 PCR Reaction (70 µL)
CUT&Tag DNA (or any tagmentated DNA)	30 µL
CUT&Tag PCR Master Mix	35 µL
CUT&Tag Index 7 Primer for Illumina Systems (10 µM)	2.5 µL
CUT&Tag Index 5 Primer for Illumina Systems (10 µM)	2.5 µL

NOTE: It is critical to change tips between tubes to avoid cross-contamination. If starting with less than 30 µL of DNA template, add DNAse-free water to bring the volume up to 30 µL.

2. Thoroughly mix the reaction by pipetting up and down and perform a quick spin to collect all liquid from the sides of the tube or plate well.

NOTE: It is critical to change tips between samples to avoid cross-contamination.

3. Place the tube on a thermocycler with a heated lid and perform PCR amplification using the following PCR cycling conditions:

a. Gap Filling 58°C for 5 min

b. Gap Filling Extension 72°C for 5 min

c. Initial Denaturation 98°C for 30 sec

d. Denaturation 98°C for 10 sec

e. Anneal and Extension 60°C for 11 sec

- For between 20,000 and 100,000 cells per CUT&Tag reaction, repeat steps d and e for a total of 13 cycles.

- For 20,000 and less cells per CUT&Tag reaction, repeat steps d and e for a total of 14-16 cycles.

NOTE: Excessive PCR cycles lead to lower library diversity and/or higher duplication rate of NGS reads.

f. Final Extension 72°C for 1 min

g. Hold 4°C

Proceed to Cleanup of PCR Amplification (Section II). (Safe Stop). Alternatively, samples can be stored at -20°C.

CUT&Tag PCR Master Mix Protocol (continued)

II. Cleanup of PCR Amplification

Before starting:

- If using AMPure XP beads, allow the beads to warm to room temperature for at least 30 minutes before use.
- Resuspend AMPure XP Beads or SPRIselect beads by tube inversion or pipetting up and down.
- Prepare 400 µL of 80% ethanol for each sample.
- Prepare approximately 20 µL of 10 mM Tris-HCl (pH 8.0-8.5) for each sample.

1. Add 70 µL (1.0X) resuspended AMPure XP beads or SPRIselect beads to 70 µL PCR reaction from Step 3 in Section I. Mix well by pipetting up and down at least 10 times. Be careful to expel all of the liquid out of the tip during the last mix.
2. Incubate samples on bench top for at least 5 minutes at room temperature.
3. Place the tube/plate on an appropriate magnetic stand for 5 minutes to separate the beads from the supernatant.
4. Carefully remove and discard the supernatant. Be careful to remove all liquid residues but not to disturb the beads that contain DNA targets.
5. Add 200 µL freshly prepared 80% ethanol to the tube/plate while in the magnetic stand. Incubate at room temperature for 30 seconds, and then carefully remove and discard the supernatant. Be careful not to disturb the beads that contain DNA targets.
6. Repeat Step 5 once for a total of two washes. Be sure to remove all visible liquid after the second wash.
7. Air dry the beads for up to 5 minutes while the tube/plate is on the magnetic stand with the lid open.

   NOTE: Do not over-dry the beads. This may result in lower recovery of DNA targets. Elute the samples when the beads are still glossy looking, but when all visible liquid has evaporated. If the beads start to crack, they are too dry.
8. Remove the tube/plate from the magnetic stand. Elute the DNA target from the beads by adding 17 µL of 10 mM Tris-HCl (pH 8.0-8.5) per sample. Mix well by pipetting up and down 10 times. Incubate for at least 2 minutes at room temperature.
9. Place the tube/plate on the magnetic stand and wait for 5 minutes. Carefully transfer 15 µL of supernatant containing the DNA targets to a new tube. (**SAFE STOP**) DNA libraries can be stored at -20°C until further use.
10. Measure the concentration of library DNA.

    NOTE: The yield of the amplified CUT&Tag DNA library can vary based on the DNA quantification method used. If using the Nanodrop or QIAxpert Systems, the expected reading is 10-20 ng/µl for histone targets and 5-12 ng/µl for non-histone targets. If the library concentration is lower than 3 ng/µl with the Nanodrop or QIAxpert Systems, please refer to the troubleshooting guide before sequencing your samples. If using the Qubit Fluorometric Quantification system or the Picogreen assay, the expected reading is 3-10 ng/µl for histone targets and could be lower than 1 ng/µl for non-histone targets.
11. Determine the size distribution of CUT&Tag DNA libraries using Agilent Bioanalyzer or TapeSatation systems, according to the manufacturer's instructions.

    NOTE: While CUT&Tag DNA libraries generated for histone modifications typically show robust signal in Bioanalyzer or TapeStation systems analysis, libraries generated for non-histone proteins such as transcription factors and cofactors often have very weak or even no visible signal using Bioanalyzer or TapeStation systems, but still generate NG-sequencing results with high mapping rates, high numbers of identified binding peaks, and acceptable signal-to-noise ratios across the whole genome. Therefore, we recommend sequencing DNA library preps from transcription factor and cofactor CUT&Tag reactions that do not show a signal in Bioanalyzer or TapeStation systems analysis.
12. Adjust the concentration of final purified library samples with 10mM Tris-HCl (pH 8.0-8.5) for high throughput sequencing. Refer to Illumina sequencing manual for optimal concentration and volume of library DNA required for NG-seq.

    NOTE: Usually the CUT&Tag DNA libraries from histone targets have a higher concentration than those from non-histone targets. We use the following formula to convert a library concentration from ng/µL to nM before diluting each library sample to the same concentration (nM) for pooling purposes: Concentration (nM) = 1,000,000 X Concentration (ng/µL) / library average size (bp) / 660. For CUT&Tag libraries where the Bioanalyzer or TapeStation system is unable to identify the average size of the library, we suggest using a size of 900 bp to intentionally pool more low-yield libraries than normal-yield libraries. In addition, we would also suggest pooling the libraries that have a flat signal of 5-10 fold more than the libraries that show normal sized peaks on the Bioanalyzer or TapeStation systems. This ensures an even distribution of the number of reads among all samples. Usually, a library pool concentration of 2 nM DNA is enough for NGS purposes, although a higher concentration is always welcome.