Analyses of Epigenetic Marks and Mechanisms in Disease: Your Guide to a Successful CUT&RUN Assay

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ABSTRACT

Like the chromatin immunoprecipitation (ChIP) assay, Cleavage Under Targets & Release Using Nuclease (CUT&RUN) is a powerful and versatile technique used for probing protein-DNA interactions within the natural chromatin context of the cell. The CUT&RUN assay can be combined with downstream qPCR or NG-seq to analyze histone modifications and binding of transcription factors, DNA replication factors, or DNA repair proteins at specific target genes or across the entire genome.

CUT&RUN provides a rapid, robust, and true low cell number assay for detection of protein-DNA interactions in the cell. Unlike the ChIP assay, CUT&RUN is free from formaldehyde cross-linking, chromatin fragmentation, and immunoprecipitation. Previously, we have shown that, compared to ChIP, CUT&RUN requires fewer starting cells (100K), has a much faster protocol (one day from cells to DNA), generates lower background signal (requires less sequencing depth), and offers spike-in control DNA for effective normalization of signal between samples and between experiments. We recently updated the CST CUT&RUN Assay Kit for use with 5,000-20,000 cells and added protocols for fixed cells and tissue.

I will discuss the basics of the CUT&RUN assay and important factors to consider when setting up your experiment. In addition, I will provide data showing the versatility of this assay for mapping various histone modifications, transcription factor, and transcription cofactor binding across multiple sample types. Finally, I will discuss how the general protocol is optimized for greater signal to noise ratio, reduced number of starting cells, and provide an alternative digestion method to prepare the input DNA as a critical control of the CUT&RUN experiment.

DESCRIPTION

The CUT&RUN Assay Kit is designed to conveniently provide reagents needed to perform up to 24 digestion reactions from cells and is optimized for 5,000-100,000 cells per reaction. The kit has been optimized to work for all types of DNA binding proteins, including histones, transcription factors and cofactors. This kit has also been validated for fixed cells, primary cells, and tissues. A complete assay can be performed in as little as one day.

The CUT&RUN Assay Kit also provides important controls to ensure a successful CUT&RUN experiment. The kit contains a positive control Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751 and a negative control Rabbit (DA1E) mAb IgG XP® Isotype Control (CUT&RUN) #66362, both of which can be used for qPCR or Next Generation sequencing (NG-seq) analysis. PCR primer sets are provided for the human (#7014) and mouse (#7015) RPL30 gene locus to be used in conjunction with the control antibodies. Tri-methyl-histone H3 Lys4 is found at the active RPL30 gene promoter, so digestion targeted by this anti- body will enrich for the RPL30 gene, while digestion targeted by the normal rabbit IgG mouse mAb will not enrich for the RPL30 gene. This kit is compatible with both qPCR and NG-seq.

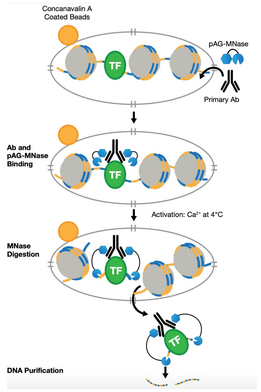
FEATURED CST REAGENTS

CUT&RUN Assay Kit #86652
CUT&RUN pAG-MNase and Spike-In DNA #40366
DNA Purification Buffers and Spin Columns (ChIP and CUT&RUN) #14209
SimpleChIP® Universal qPCR Master Mix #88989
DNA Library Prep Kit for Illumina® (ChIP-seq, CUT&RUN) #56795
Multiplex Oligos for Illumina® (Dual Index Primers) (ChIP-seq, CUT&RUN) #47538
Multiplex Oligos for Illumina® (Single Index Primers) (ChIP-seq, CUT&RUN) #29580

REFERENCES

1. Skene, J.S. and Henikoff, S. (2017) *eLIFE* 6, e21856.
2. Skene, J.S. et al. (2018) *Nature Protocols* 13, 1006-1019.
3. Meers, M.P. et al. (2019) *eLIFE* 8, e46314.
4. Meers, M.P. et al. (2019) *Molecular Cell* 75, 1-14.

CUT&RUN Method Overview



Antibody and pAG-MNase Binding:

1. Cells are immobilized on Concanavalin A Magnetic Beads to allow for subsequent buffer and reagent exchanges.
2. Cell membranes are then permeabilized with digitonin to facilitate the entry of primary antibody and pAG-MNase fusion enzyme into the cell nuclei.
3. The target-specific primary antibody recruits the pAG-MNase to the chromatin through protein-protein interactions between the antibody and the pAG domain of the fusion enzyme.

MNase Digestion:

4. The addition of Ca2+ activates the pAG-MNase, which gently cleaves and liberates the desired chromatin fragments, allowing them to diffuse away from the genomic chromatin, out of the cell, and into the supernatant.

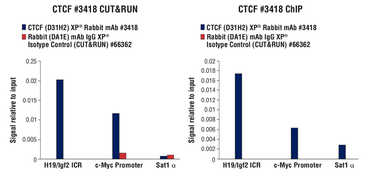
DNA Purification:

5. DNA is purified using DNA purification spin columns or phenol/chloroform extraction followed by ethanol precipitation. The purified, enriched DNA is then identified and quantified using qPCR or NG-seq.

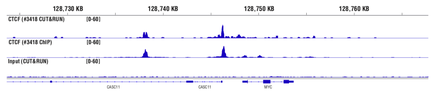
Advantages

- **Target versatility:** Generate sequencing and/or qPCR data for histones, histone modifications and low abundance/weak binding transcription factors and cofactors.
- **Low sample requirements:** 5,000 to 10,000 cells for histones. 10,000 to 20,000 cells for transcription factors and cofactors.
- **Compatible with fixed cells:** Light cell fixation keeps cells intact, preserves cell signaling pathways, and enhances the enrichment for accessory components of huge complexes.
- **Use fixed or fresh tissue samples:** Validated and optimized tissue protocols.
- **Fast time to results:** 1-2 days from cells to DNA.
- **Low sequencing cost:** Only requires 3-5 million high-quality reads per sample due to the inherently low assay background.
- ***In Vivo* method:** Uses native chromatin, eliminating cross-linking artifacts.
- **Antibody versatility:** Compatible with rabbit and mouse antibodies.
- **Straightforward quantification:** Spike in control DNA to simplify data quantification and normalization.

CUT&RUN Comparison to ChIP-seq



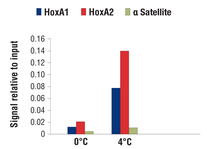
CUT&RUN and ChIP assays were performed with HCT 116 cells using the CUT&RUN Assay Kit #86652 (left panel) or SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005 (right panel). The enriched DNA was quantified by real-time PCR using human c-Myc promoter primers, SimpleChIP® Human H19/Igf2 ICR Primers #5172, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.



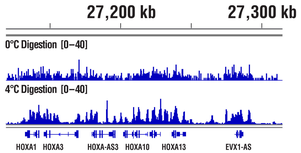
CUT&RUN and ChIP DNA libraries were prepared using DNA Library Prep Kit for Illumina® #56795. These tracks compare enrichment at the MYC gene, a known target of CTCF. The input tracks are from the CUT&RUN input sample.

Optimizing MNase Digestion Temperature

SUZ12 (D39F6) XP® Rabbit mAb #3737



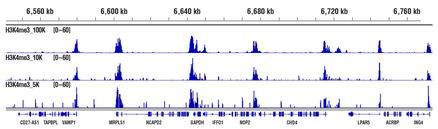
Digesting DNA at 4°C greatly improves signal to noise ratio. CUT&RUN was performed with NCCIT cells using the CUT&RUN Assay Kit #86652 and DNA was digested at 0°C or 4°C. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human HoxA1 Intron 1 Primers #7707, SimpleChIP® Human HoxA2 Promoter Primers #5517, and SimpleChIP® Human α Satellite Repeat Primers #4486.



Digesting DNA at 4°C greatly improves target specific gene enrichment. These tracks show enrichment of SUZ12 across the HoxA gene cluster.

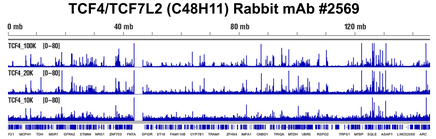
Low Cell Input for CUT&RUN

Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751



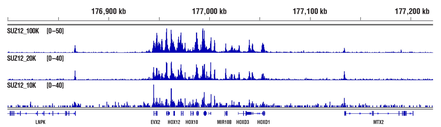
Histone modifications typically require only 5,000 cells. CUT&RUN was performed with 100,000, 10,000, or 5,000 HCT 116 cells (as indicated) using the CUT&RUN Assay Kit #86652. These tracks show enrichment around the GAPDH gene, a known target of H3K4me3.

TCF4/TCF7L2 (C48H11) Rabbit mAb #2569



Transcription factors typically require only 10,000 to 20,000 cells. CUT&RUN was performed with 100,000, 20,000, or 10,000 HCT 116 cells (as indicated) using the CUT&RUN Assay Kit #86652. These tracks show binding of TCF4/TCF7L2 across chromosome 8.

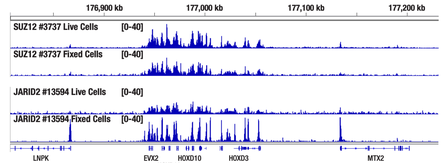
SUZ12 (D39F6) XP® Rabbit mAb #3737



Cofactors typically require only 10,000 to 20,000 cells. CUT&RUN was performed with 100,000, 20,000, or 10,000 NCCIT cells (as indicated) using the CUT&RUN Assay Kit #86652. These tracks show binding across the HoxD gene cluster, a known target of SUZ12.

Using Fixed Cells in CUT&RUN

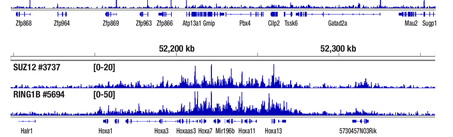
SUZ12 (D39F6) XP® Rabbit mAb #3737
JARID2 (D6M9X) Rabbit mAb #13594



Fixation is not required for SUZ12 (a core component of PRC2 complex) but increases the enrichment for JARID2 (an accessory component of PRC2 complex). CUT&RUN was performed with 100,000 live or fixed NCCIT cells (0.1% formaldehyde, 10 min) using the CUT&RUN Assay Kit #86652. These tracks show binding of SUZ12 and JARID2 across the HOXD gene cluster.

CUT&RUN is Compatible with Tissues

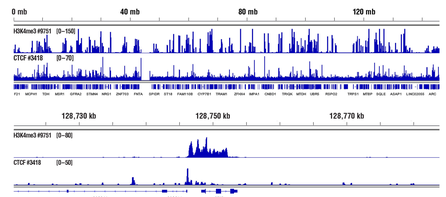
Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751
Glucocorticoid Receptor (D6H2L) XP® Rabbit mAb #12041
SUZ12 (D39F6) XP® Rabbit mAb #3737
RING1B (D22F2) XP® Rabbit mAb #5694



Tissue samples may require various fixation conditions and amount of starting material depending on tissue type. CUT&RUN was performed with 2.5 mg of medium-fixed mouse liver tissue (0.1% formaldehyde, 10 min) using the CUT&RUN Assay Kit #86652. The upper panel shows binding of H3K4me3 and Glucocorticoid Receptor around the Zfp866 gene, while the lower panel shows binding of SUZ12 and RING1B across the HoxA gene cluster.

Chromatin Profile in Primary Cells

Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751
CTCF (D31H2) XP® Rabbit mAb #3418



Primary cell model using only 100,000 cells. CUT&RUN was performed with 100,000 live human CD8+ T cells using CUT&RUN Assay Kit #86652. The upper panel shows binding across chromosome 8, while the lower panel shows enrichment around the MYC gene, a known target of H3K4me3 and CTCF.

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