



# Not enough time or sample for Chromatin IP? Try CUT&RUN!

## What is CUT&RUN?

CUT&RUN stands for **C**leavage **U**nder **T**argets & **R**elease **U**sing **N**uclease.

It is an *in vivo* method that uses a target-specific primary antibody and a Protein A-Protein G-Micrococcal Nuclease (pAG-MNase) to isolate specific protein-DNA complexes<sup>1,2,3</sup>.

### REFERENCES

1. Skene P.J., et al. (2018) Nat. Protoc. 13(5), 1006-1019.
2. Meers M.P., et al. (2019) BioRxiv 1, 569129.
3. Skene P.J., Henikoff S. (2017) Elife 6, e21865.

## CUT&RUN Products

Cell Signaling Technology offers two flexible solutions. Try our new kit with your favorite target antibody. The kit contains the pAG-MNase, all the necessary buffers and reagents, and a detailed protocol. Or just order the pAG-MNase and Spike-In DNA if you prefer.

### #86652 CUT&RUN Assay Kit

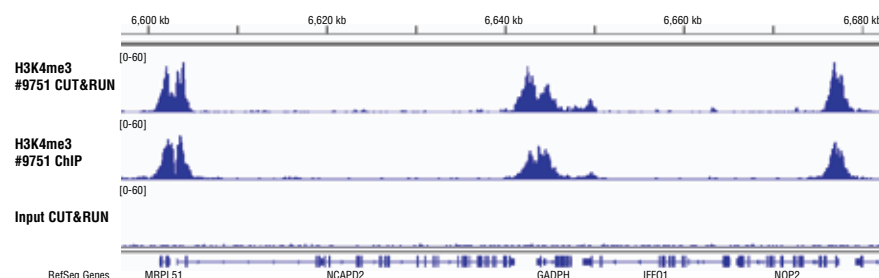
### #40366 CUT&RUN pAG-MNase and Spike-In DNA

### #14209 DNA Purification Buffers and Spin Columns (ChIP, CUT&RUN)

## The Benefits of CUT&RUN

<b>Low sample requirement</b>	100K cells recommended
<b>Fast time to results</b>	1-2 days from cell to DNA
<b>Sequencing cost savings</b>	Only 3-5 million high-quality reads required
<b>Target versatility</b>	Generate sequencing and/or qPCR data for histones, histone modifications, transcription factors, and cofactors
<b>Antibody versatility</b>	Compatible with rabbit and mouse antibodies
<b>Reproducible results</b>	Spike-In control DNA to normalize signal between samples
<b>Avoid "cross linking" artifacts</b>	An <i>in vivo</i> method performed using native chromatin

## The CUT&RUN Assay Kit from CST is ideally recommended for use with 100,000 cells and works as well as ChIP-seq



CUT&RUN and ChIP assays were performed with HCT 116 cells ( $1 \times 10^5$  for CUT&RUN,  $4 \times 10^6$  cells for ChIP) and Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751, using the CUT&RUN Assay Kit #86652 or the SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA Libraries were prepared using DNA Library Prep Kit for Illumina (ChIP-seq, CUT&RUN) #56795. Comparison of enrichment at the GAPDH gene, a known target of H3K4me3. The input track is from the CUT&RUN input sample.

For additional information, please visit:  
[www.cst-science.com/CUT-RUN](http://www.cst-science.com/CUT-RUN)

# How CUT&RUN Works

## Method Overview

### Ab & pAG-MNase binding:

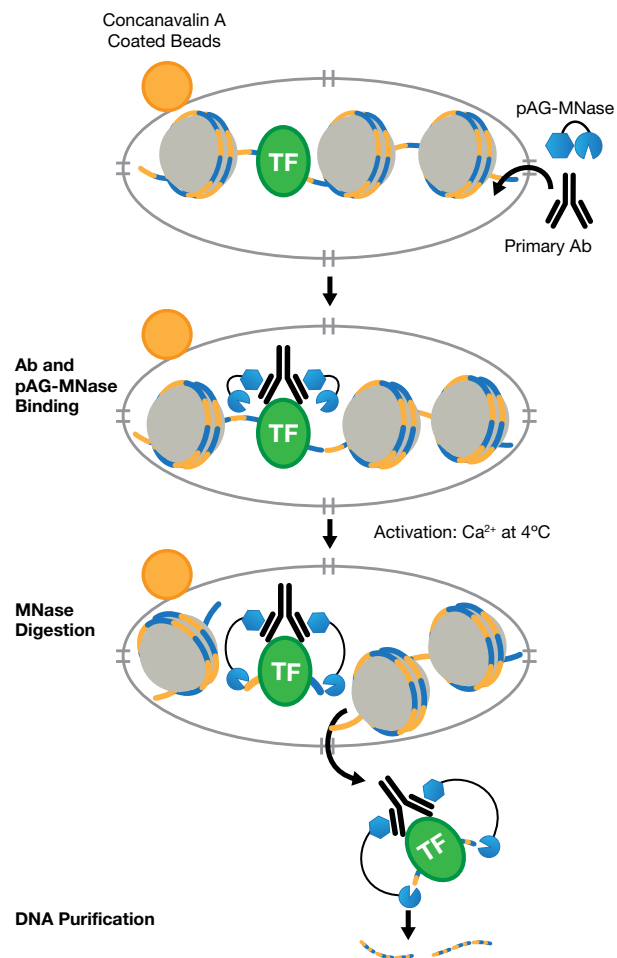
1. Cells are immobilized on Concanavalin A Magnetic Beads to allow for subsequent buffer and reagent exchanges.
2. Cell membranes are then permeabilized with digitonin to facilitate the entry of primary antibody and pAG-MNase fusion enzyme into the cell nuclei.
3. The target-specific primary antibody 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  $\text{Ca}^{2+}$  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.



## Contact Information

Technical Support: [www.cellsignal.com/support](http://www.cellsignal.com/support)

Ordering Information: [www.cellsignal.com/orderinfo](http://www.cellsignal.com/orderinfo)

For a complete list of CST offices and distributors, please visit:  
[www.cellsignal.com/contactus](http://www.cellsignal.com/contactus)