Store at -20°C

CUT&RUN pAG-MNase and Spike-In DNA

#40366

50 assays

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

For Research Use Only. Not For Use In Diagnostic Procedures.

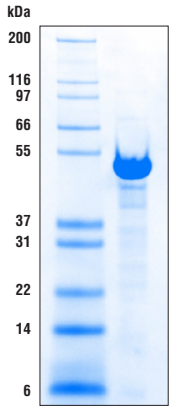
Products Included	Item #	Quantity	Number in Kit
pAG-MNase Enzyme	57813	40 µl	2
Sample Normalization Spike-In DNA (10 pg/µl)	29987	250 µl	1

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The CUT&RUN pAG-MNase Enzyme and Spike-In DNA kit provides enough enzyme and spike-in DNA to support 50 CUT&RUN assays. The pAG-MNase Enzyme #57813 is a fusion of Protein A and Protein G to Micrococcal Nuclease, and is recombinantly produced in *E. coli*. The pAG-MNase is compatible with multiple species of antibodies, including both rabbit and mouse. Sample Normalization Spike-In DNA (10 pg/µl) #29987 is fragmented genomic DNA from the yeast *S. cerevisiae* that can be added to a CUT&RUN reaction to facilitate normalization between samples during NG-seq analysis.

Background: Like the chromatin immunoprecipitation (ChIP) assay, Cleavage Under Targets and 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 (1-4). 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, making it a much faster and more efficient method for enriching protein-DNA interactions and identifying target genes. CUT&RUN can be performed in less than one day, from live cells to purified DNA, and has been shown to work with as few as 500-1000 cells per assay (1,2). Instead of fragmenting all of the cellular chromatin as done in ChIP, CUT&RUN utilizes an antibody-targeted digestion of chromatin, resulting in much lower background signal than seen in the ChIP assay. As a result, CUT&RUN requires only 1/10th the sequencing depth that is required for ChIP-seq assays (1,2). Finally, the inclusion of simple spike-in control DNA allows for accurate quantification and normalization of target-protein binding that is not possible with the ChIP method. This provides for effective normalization of signal between samples and between experiments.

Background References: Background references are listed on the last page of this data sheet.



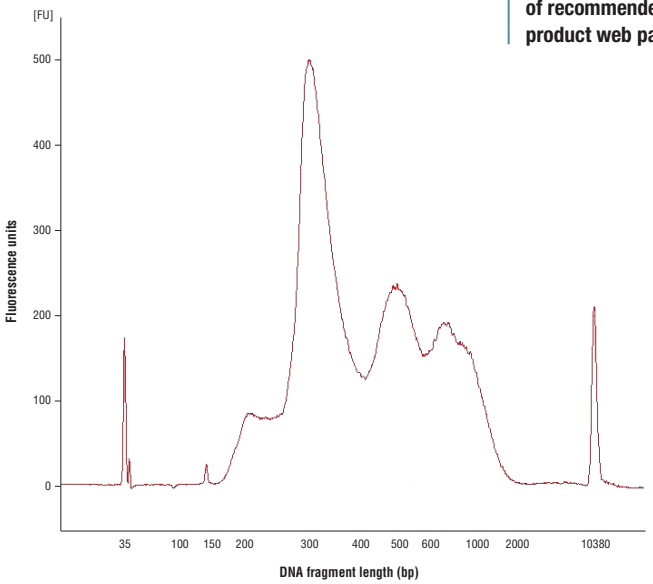
pAG-MNase was expressed and purified from E. coli. Purified enzyme was resolved on an SDA-PAGE gel and stained with Coomassie blue. The molecular weight of the protein standards is indicated.

Storage: pAG-MNase Enzyme is supplied in 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1mM EDTA, 0.1mM PMSF and 50% glycerol. *Do not aliquot the product.* Sample Normalization Spike-In DNA is supplied in 10 mM Tris-HCl (pH 8.0). Store both products at –20°C. *These products are stable for at least 12 months.*

Directions for Use: For the pAG-MNase Enzyme, after cell permeabilization and primary antibody binding, resuspend cells in 50 µl of binding buffer containing 1.5 µl of pAG-MNase Enzyme (33X dilution). Incubate cell samples with rotation at 4°C for 1 hour, wash cells with binding buffer, and then perform the chromatin digestion.

Sample Normalization Spike-In DNA can be added directly to the digestion stop buffer. For sample normalization with NG-seq, add 5 µl (50 pg) of Sample Normalization Spike-In DNA to each reaction. When using 100,000 cells or 1mg of tissue per reaction this ensures that the normalization reads are around 0.5% of the total sequencing reads. If more or less than 100,000 cells or 1mg of tissue are used per reaction, proportionally scale the volume of Sample Normalization Spike-In DNA up or down to adjust normalization reads to around 0.5% of total reads. When performing sample normalization, be sure to map the CUT&RUN sequencing data for all samples to both the test reference genome (e.g. human) and the sample normalization genome (*S. cerevisiae*).

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.



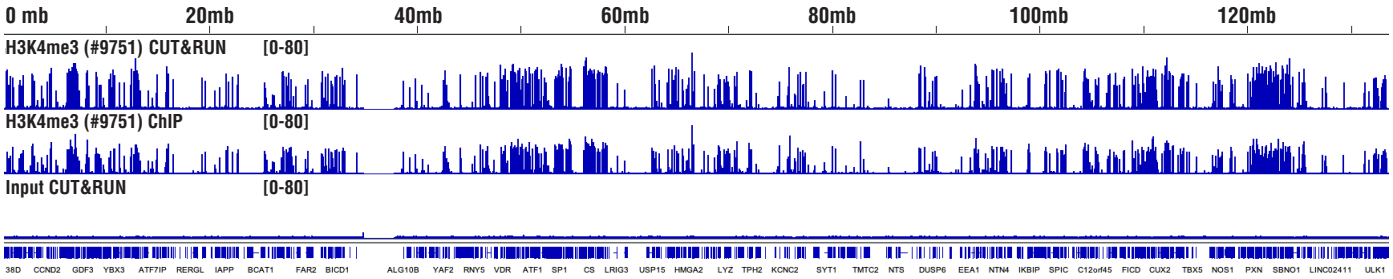
CUT&RUN assays were performed with HCT 116 cells and Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. The size of the DNA fragments in the library were analyzed using an Agilent Bioanalyzer®. The adaptor and barcode sequences added to the library during construction account for 140 bp in fragment length. As shown, excised DNA is highly enriched for mononucleosomes (peak around 300 bp).

Thank you for your recent purchase. If you would like to provide a review visit cellsignal.com/comments.

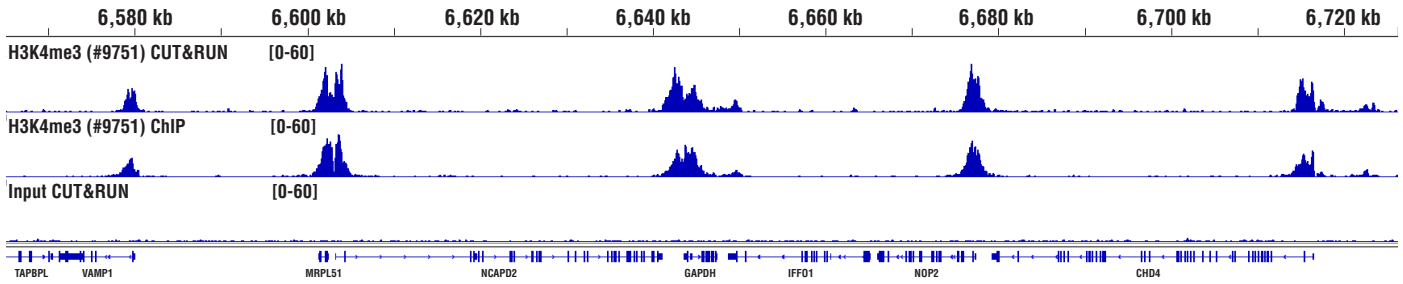
www.cellsignal.com

Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **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.

Panel A

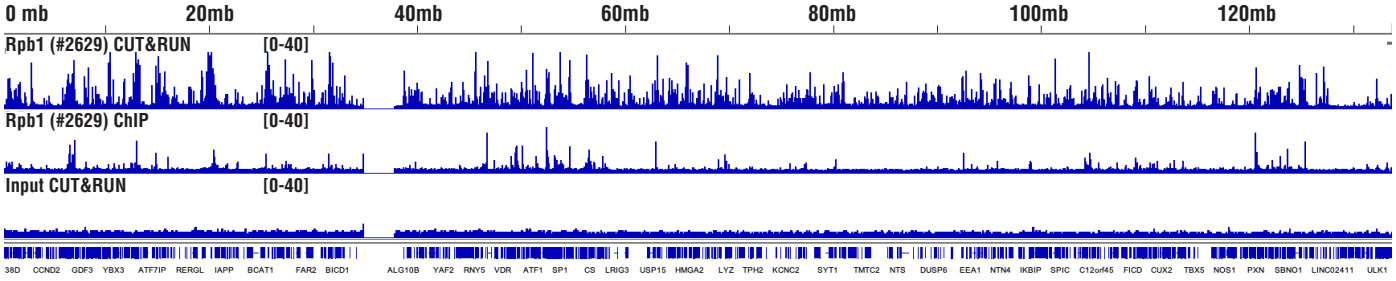


Panel B

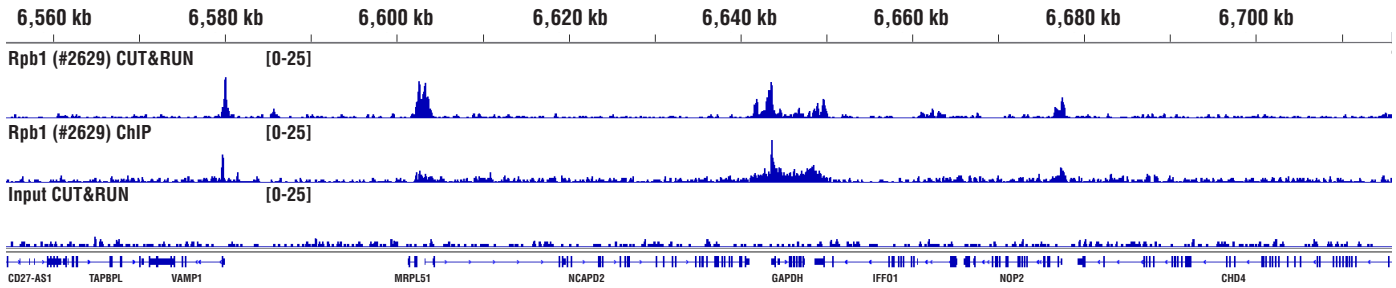


CUT&RUN and ChIP assays were performed with HCT 116 cells and Tri-Methyl-Histone H3 (Lys4) (C42D8) Rabbit mAb #9751. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. Panel A compares enrichment of H3K4me3 across chromosome 12 (upper), while Panel B compares enrichment at the GAPDH gene (lower), a known target of H3K4me3. The input tracks are from the CUT&RUN input sample.

Panel A

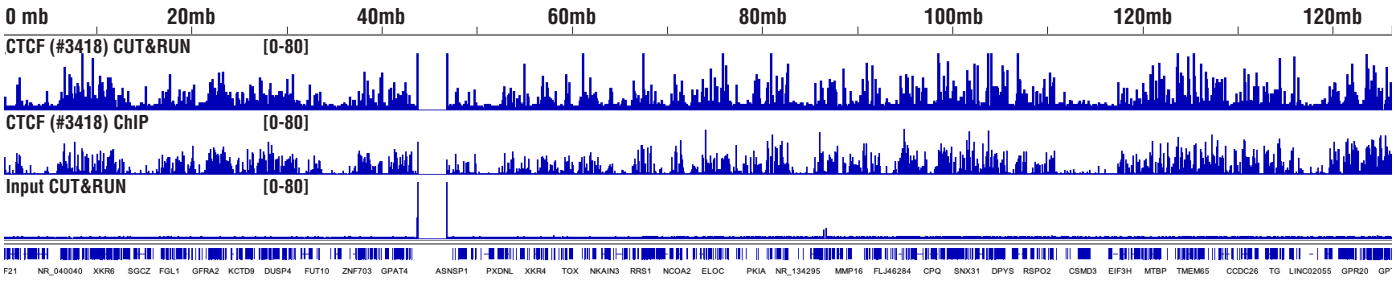


Panel B

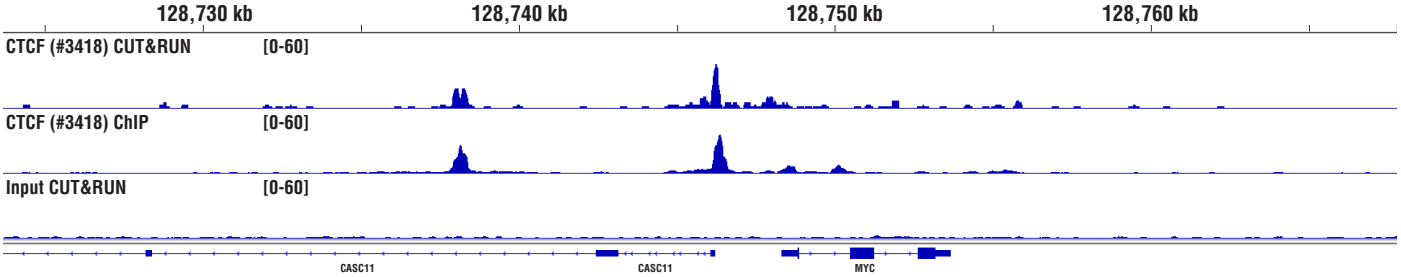


CUT&RUN and ChIP assays were performed with HeLa cells and Rpb1 CTD (4H8) Mouse mAb #2629. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. Panel A compares enrichment of Rpb1 across chromosome 12 (upper), while Panel B compares enrichment at the GAPDH gene (lower), a known target of Rbp1. The input tracks are from the CUT&RUN input sample.

Panel A



Panel B



CUT&RUN and ChIP assays were performed with HCT 116 cells and CTCF (D31H2) XP® Rabbit mAb #3418. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. Panel A compares enrichment of CTCF across chromosome 8 (upper), while Panel B compares enrichment at the MYC gene (lower), a known target of CTCF. The input tracks are from the CUT&RUN input sample.

Background References:

(1) Skene, P.J. and Henikoff, S. (2017) *Elife 6, pii: e21856. doi: 10.7554/eLife.21856.*

(2) Skene, P.J. et al. (2018) *Nat Protoc* 13, 1006-19.

(3) Meers, M.P. et al. (2019) *Elife 8, pii: e46314. doi: 10.7554/eLife.46314.*

(4) Meers, M.P. et al. (2019) *Mol Cell* 75, 562-575.e5.