Store at -20°C

Digitonin Solution

#16359

2 x 1.2 ml

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New 06/20

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

Description: The Digitonin Solution provides enough reagent to support 24 CUT&RUN assays. This product is formulated for optimal performance in the CUT&RUN assay and each lot is tested and validated using the CUT&RUN Assay Kit #86652. This product should be completely thawed at 90-100°C for 5 minutes before using. Please keep on ice during use and store at -20°C when finished for the day.

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.

Storage: Digitonin Solution is supplied in nuclease-free water. Store at -20°C. *This product is stable for at least 12 months.*

Directions for Use: For the CUT&RUN assay, we recommend adding 25 µl of Digitonin Solution per 1 ml buffer. Not all cell lines exhibit the same sensitivity to digitonin, so it may be necessary to adjust the final volume of digitonin in the buffer to get optimal permeabilization of your specific cell line. Cell permeabilization can be tested by combining an equal volume of cell suspension and 0.4% Trypan Blue Stain. Optimal cell permeabilization will result in staining of >90% of the cell population.

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.

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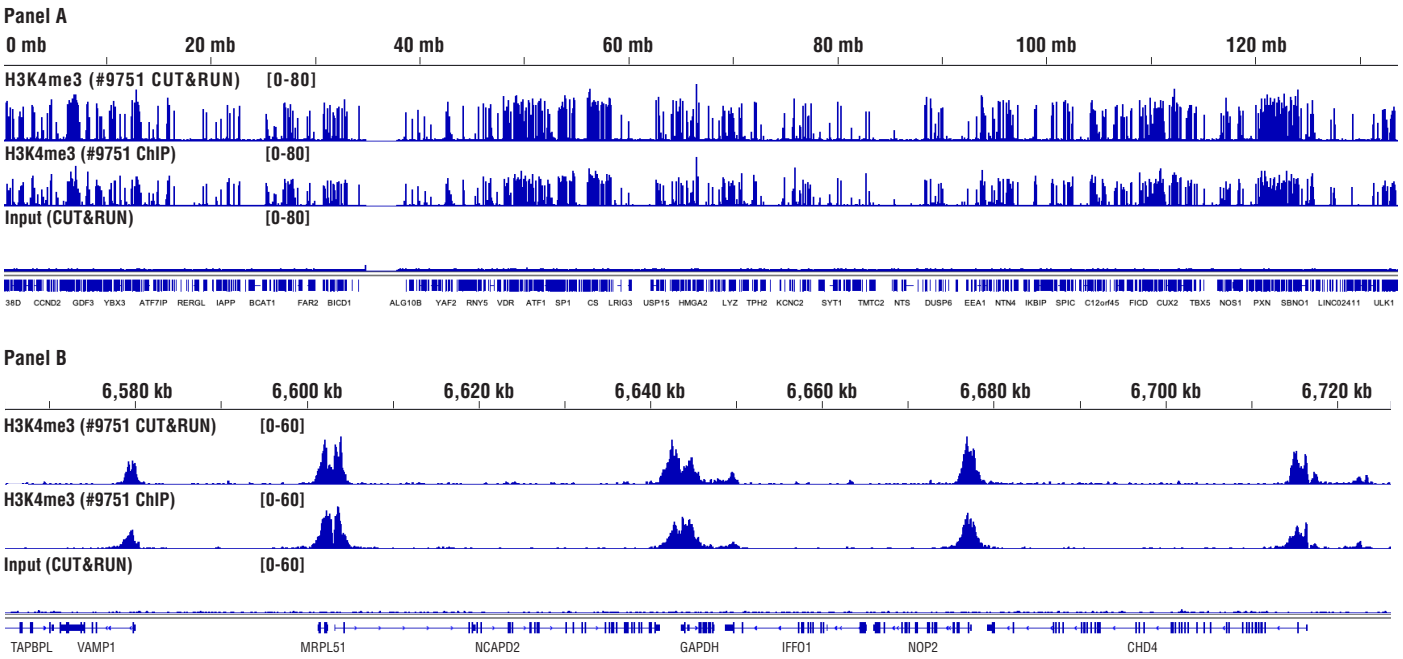
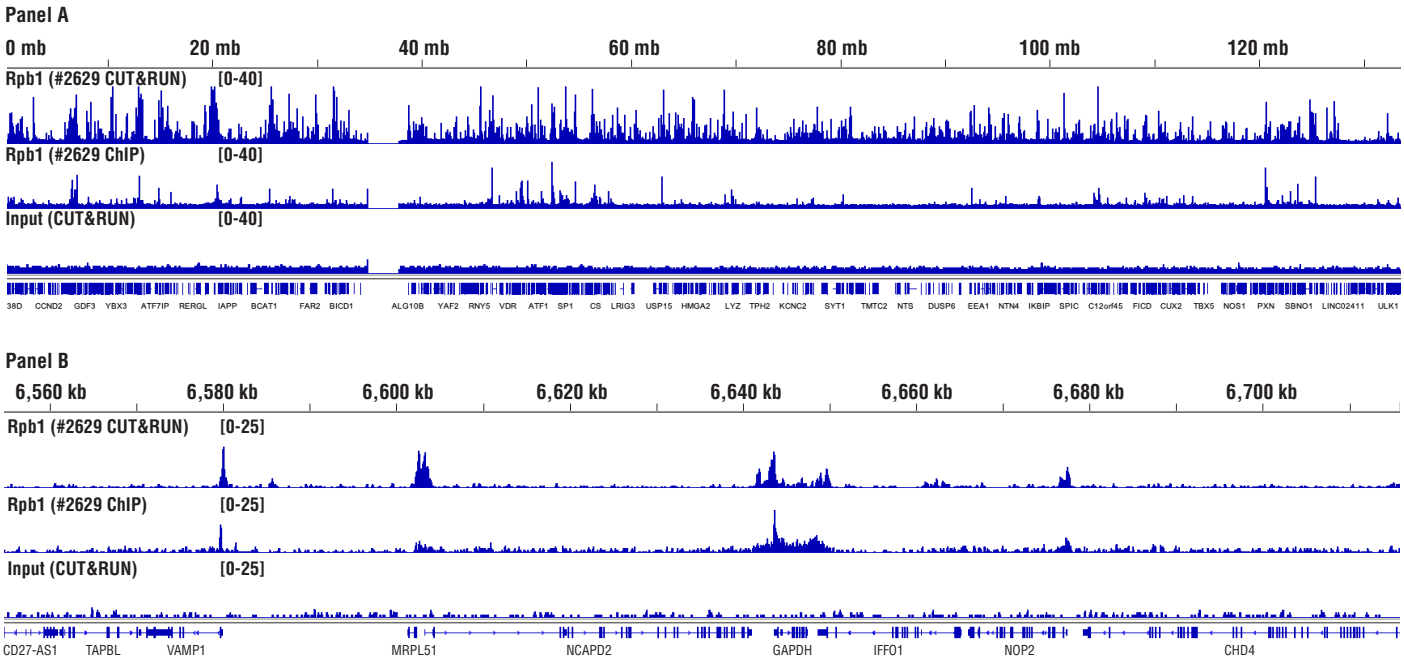
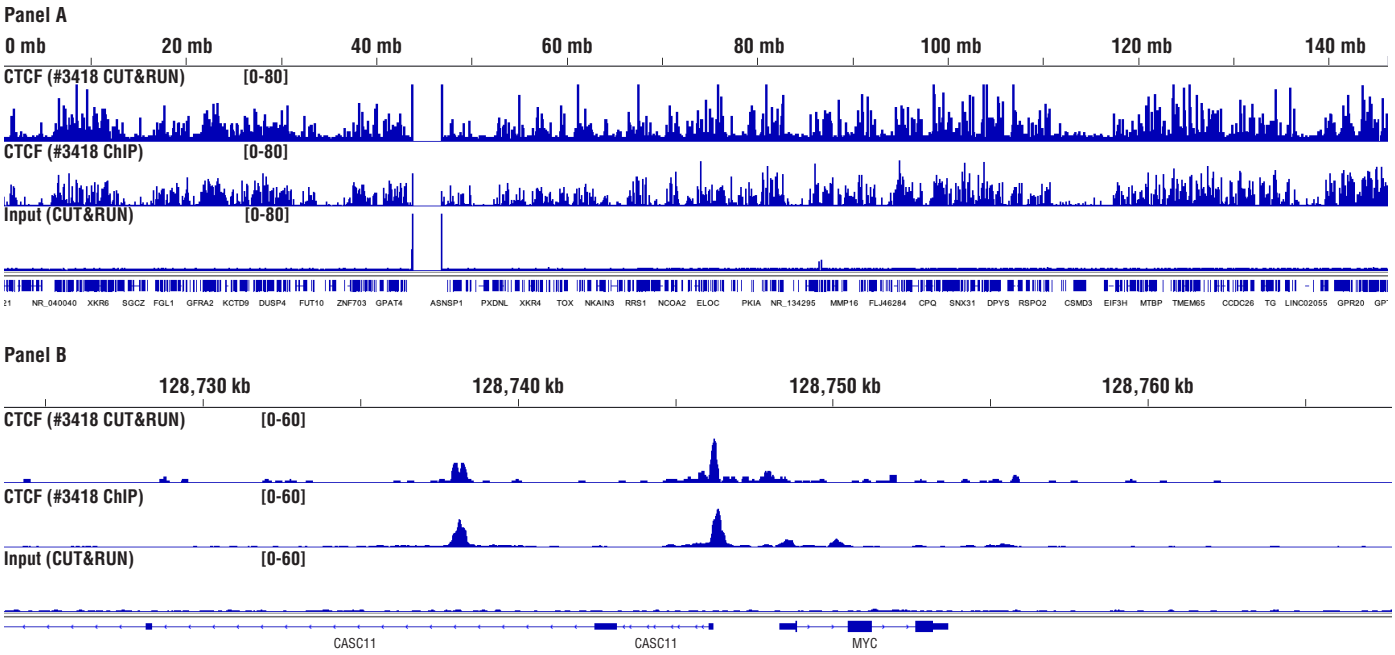
Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **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.



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.



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 Rpb1. The input tracks are from the CUT&RUN input sample.



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.