

Store at
-20°C

CUT&RUN 4X Stop Buffer

#48105

1 mL



Cell Signaling
TECHNOLOGY®

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New 02/21

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

Description: The CUT&RUN 4X Stop Buffer 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 diluted to 1X using nuclease-free water and an appropriate amount of Digitonin Solution #16359 and RNase A (10 mg/ml) #7013 should be added right before use. Addition of Spike-In DNA is optional.

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 500-1,000 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: Store CUT&RUN 4X Stop Buffer at -20°C. This product is stable for at least 12 months.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Directions for Use: For the CUT&RUN assay, we recommend adding 37.5 µl CUT&RUN 4X Stop Buffer, 3.75 µl Digitonin Solution #16359, and 0.75 µl RNase A (10 mg/ml) #7013 to 108 µl nuclease-free water (150 µl per reaction) right before use. Then incubate with the sample at 37°C for 10 min. An appropriate amount of Sample Normalization Spike-In DNA can be added to CUT&RUN 4X Stop Buffer, if desired.

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.