Store at 4°C

10X Wash Buffer (CUT&RUN, CUT&Tag)

#31415

15 mL

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not for Use in Diagnostic Procedures.

Description: The 10X Wash Buffer (CUT&RUN, CUT&Tag) provides enough reagent to support 24 CUT&RUN or CUT&Tag assays. This product is formulated for optimal performance in the CUT&RUN and CUT&Tag assays and each lot is tested and validated using the CUT&RUN Assay Kit #86652 or the CUT&Tag Assay Kit #77552. This product should be diluted to 1X using nuclease-free water and an appropriate amount of 100X Spermidine #27287 and Protease Inhibitor Cocktail (200X) #7012 should be added right before use. Please keep at room temperature during use to minimize stress on the cells.

Background: Like the chromatin immunoprecipitation (ChIP) assay, Cleavage Under Targets and Release Using Nuclease (CUT&RUN) and Cleavage Under Targets and Tagmentation (CUT&Tag) are powerful and versatile techniques used for probing protein-DNA interactions within the natural chromatin context of the cell (1-7). 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 signals between samples and between experiments. CUT&Tag has many of the same advantages as the CUT&RUN assay in that it provides a rapid, robust, and true low cell number protocol for detection of protein-DNA interactions in the cell. In addition, the CUT&Tag assay adds an in situ adaptor DNA ligation step carried out by the pAG-Tn5 enzyme, in which an adaptor DNA is ligated directly to antibody-targeted chromatin DNA fragments in the cell. As a result, subsequent DNA library preparation is much faster and easier than library preparation following the CUT&RUN assay, free from DNA end repair, A-tailing, and adaptor ligation in vitro. CUT&Tag works very well for analyzing histone modifications, in addition to mapping some transcription factors and cofactors binding.

Storage: Store 10X Wash Buffer (CUT&RUN, CUT&Tag) at 4°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 and CUT&Tag assays, we recommend preparing 2 ml 1X Complete Wash Buffer for each cell line and an additional 100 µl for each reaction or input sample. For example, to prepare 2.5 ml of 1X Complete Wash Buffer, add 250 µl 10X Wash Buffer (CUT&RUN, CUT&Tag), 25 µl 100X Spermidine #27287, and 12.5 µl Protease Inhibitor Cocktail (200X) #7012 to 2,212.5 µl nuclease-free water right before use. Equilibrate it to room temperature to minimize stress on the cells.

Background References:

(1) Skene, P.J. and Henikoff, S. (2017) *Elife* 6, .
(2) Skene, P.J. et al. (2018) *Nat Protoc* 13, 1006-1019.
(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.
(5) Kaya-Okur, H.S. et al. (2019) *Nat Commun* 10, 1930.
(6) Kaya-Okur, H.S. et al. (2020) *Nat Protoc* 15, 3264-3283.
(7) Henikoff, S. et al. (2021) *Bio Protoc* 11, e4043.

Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **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.