

Store at
4°C

Antibody Binding Buffer (CUT&RUN, CUT&Tag)

#15338

2 x 2.5 mL

**Cell Signaling**
TECHNOLOGY®**Support:** +1-978-867-2388 (U.S.)
cellsignal.com/support**Orders:** 877-616-2355 (U.S.)
orders@cellsignal.com

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

Description: The Antibody Binding Buffer (CUT&RUN, CUT&Tag) provides enough reagent to support 48 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. An appropriate amount of Digitonin Solution #16359, 100X Spermidine #27287, and Protease Inhibitor Cocktail (200X) #7012 should be added to this product right before use. Please keep on ice during use.

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 Antibody Binding 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 or CUT&Tag assay, we recommend adding 1 µL 100X Spermidine #27287, 0.5 µL Protease Inhibitor Cocktail (200X) #7012, and 2.5 µL Digitonin Solution #16359 to 96 µL Antibody Binding Buffer (CUT&RUN, CUT&Tag) (100 µL per reaction) right before use. Please keep on ice during use. The volume of Digitonin Solution #16359 in the Antibody Binding Buffer (CUT&RUN, CUT&Tag) can be adjusted based on the specific cell line.

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, .
- (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.

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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.