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96-well plate CUT&RUN (BC 22.11.04)

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ABSTRACT

CUT&RUN offers a convenient and practical means to perform chromatin profiling *in situ* for low cell number samples. This well plate format version of the assay has been adapted from the Rudensky lab (van Der Veeken et al. Immunity. 2020.

PROTOCOL CITATION

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Procedure

1 Prepare buffers prior to starting. These buffers work optimally for T cells:

Buffer 1 (1x perm buffer from eBioscience Foxp3/TF staining buffer set diluted in nuclease free water, 1X EDTA-free protease inhibitors, 0.5mM spermidine)

A	B	C
Component	Amount	Final Concentration
10X permeabilization buffer	1mL	1X
50X cOmplete inhibitor	200uL	1X
1.3M spermidine	3.85uL	0.5mM
Nuclease free water	8.796mL	

Recipe for 10ml. Scale up as needed.

Antibody buffer: Buffer 1 + 2mM EDTA

Buffer 2 (0.05% saponin, 1X EDTA-free protease inhibitors, 0.5mM spermidine in PBS)

A	B	C
Component	Amount	Final Concentration
5% saponin (100X)	100uL	0.05%
50X cOmplete inhibitor	200uL	1X
1.3M spermidine	3.85uL	0.5mM
PBS	9.696mL	

Recipe for 10ml. Scale up as needed.

Calcium buffer: Buffer 2 + 2mM CaCl2

2X Stop buffer: 20mM EDTA, 4mM EGTA in buffer 2

1.1 If using a non-T cell cell type, you can substitute in buffers from the Henikoff protocol (https://www.protocols.io/view/cut-amp-run-targeted-in-situ-genome-wide-profiling-14egnr4ql5dy/v3)

In limited attempts, I have had successful runs substituting in Henikoff's antibody buffer for initial wash and primary antibody staining steps, and using Henikoff's digi-wash buffer for subsequent wash steps, MNase incubation, and calcium activation steps. After you determine the optimal digitonin concentration for your cell type, there is a possibility that these buffer condtions can work well when used in this plate protocol.

2 Harvest 100,000 to 500,000 cells per replicate and transfer each sample to individual wells of a

V-bottom 96 well plate (max well volume ~ **200 µL**). I use Nunc MicroWell plates (Thermo Scientific Cat #249944), however any V-bottom plate should work fine.

3 Centrifuge plate for the equivalent of 1900rpm, **00:06:00** on Sorvall Legend XTR centrifuge (need to convert to rcf). Use this same speed and duration for all subsequent centrifugation steps. 6m

4 Carefully remove supernatant with a P200, and add **200 µL** of antibody buffer to wash the cell pellet. If using >=100k cells, cell pellet should be clearly visible. For each subsequent wash step, use **200 µL** of specified buffer.

5 Spin down plate and remove antibody buffer wash.

6 Incubate cells with antibodies in **100 µL** of antibody buffer for 1h on ice.

7 Spin down plate and remove antibody solution. Wash samples twice with buffer 1. You can add additional wash steps, however I have not observed an advantage in doing so.

8 Incubate samples with pA/G-MNase (20X) in buffer 1 for **01:00:00** at **4 °C** . Use **50 µL** buffer 1 for each sample (**2.5 µL** of pA/G-MNase per sample) Epicypher's CUTANA makes a good enzyme (EpiCypher SKU: 15-1016) 1h

9 Spin down plate and remove enzyme solution. Wash samples twice in buffer 2 (**200 µL** for each wash), then resuspend samples in **100 µL** of calcium buffer to activate the MNase. Incubate the samples for **00:30:00** on wet ice. 30m

10 Following incubation in calcium buffer, add **100 µL** of 2X stop solution. Incubate samples for **00:15:00** in a **37 °C** incubator to release cleaved chromatin fragments. Incubation time can be adjusted, but 10-20m is recommended to avoid over/under releasing fragments. 15m

Collect supernatant containing target chromatin fragments by centrifugation (1900rpm, 6min)

11 and extract DNA. Qiagen MinElute kits are fine, but will exclude fragments below 70bp. CST offers alternative column kits that retain smaller fragments.

12 Use Nan Liu's library prep protocol to prepare CUT&RUN libraries: Nan Liu's C&R Library Prep Protocol