CUTANA™ ChIC / CUT&RUN Kit

QUICK-START CARD

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DAY 1

Section I: CUT&RUN Buffer Preparation (~30 min)

1. Prepare buffers as outlined in the Table below. **Note:** The amount of Digitonin should be optimized for each cell type as instructed in **Appendix 1.1** of the manual.

BUFFER NAME	COMPONENTS	1 RXN	8 RXN	16 RXN	STORAGE
Wash Buffer	Pre-Wash Buffer	1.8 mL	14.4 mL	28.8 mL	RT for use on Day 1
	25X Protease Inhibitor	72 µL	576 µL	1.15 mL	
	1 M Spermidine	0.9 µL	7.2 µL	14.4 µL	
Cell Permeabilization Buffer	Wash Buffer	1.4 mL	11.2 mL	22.4 mL	4°C for use on Day 2
	5% Digitonin	2.8 µL	22.4 µL	44.8 µL	
Antibody Buffer	Cell Perm. Buffer	100 µL	800 µL	1.6 mL	Ice for use on Day 1
	0.5 M EDTA	0.4 µL	3.2 µL	6.4 µL	

Section II: ConA Bead Activation (~30 min)

2. Gently resuspend **ConA beads** and transfer 11 µL per reaction to a 1.5 mL tube.
3. Place tube on a magnet and allow slurry to clear. Pipette to remove supernatant.
4. Remove tube from magnet. Immediately add 100 µL/reaction cold **Bead Activation Buffer** and pipette to resuspend. Return tube to magnet, allow slurry to clear, and pipette to remove supernatant. Repeat one time.
5. Resuspend beads in 11 µL/reaction cold **Bead Activation Buffer**.
6. Aliquot 10 µL/reaction of beads into **8-strip tubes**. Place on ice.

Section III: Binding Cells to Activated Beads (~30 min)

7. Count starting cells and confirm integrity and viability. Harvest 500,000 cells/reaction (plus 10% excess).
8. Spin at 600 x g for 3 min at room temperature (**RT**). Remove supernatant.
9. Resuspend cells in 100 µL/reaction RT **Wash Buffer**. Spin 600 x g, 3 min, RT. Remove supernatant. Repeat one time.
10. Resuspend cells in 105 µL/reaction RT **Wash Buffer**. Count and examine integrity of prepared cells.
11. Add 100 µL cells to 10 µL **activated ConA beads** in 8-strip tubes. Gently vortex to resuspend and quick spin in a mini-centrifuge to collect beads in bottom of tubes.
12. Incubate 10 min at RT to adsorb cells to beads.
13. If using a multi-channel pipettor, place a reagent reservoir on ice. Fill with cold **Antibody Buffer**. **Note:** Remove and replace buffers one tube strip at a time to avoid ConA bead dry-out and sample loss.
14. Place tubes on a magnet, allow slurry to clear, and pipette to remove supernatant. Save 10 µL supernatant for Trypan Blue staining to confirm that cells are not in supernatant (**Appendix 1.2**).
15. Remove tubes from magnet. Immediately add 50 µL/reaction cold **Antibody Buffer** and pipette to resuspend. Take a 10 µL aliquot to confirm ConA bead binding (**Appendix 1.2**).

Section IV: Antibody Binding (~30 min + overnight)

16. Quick spin the **K-MetStat Panel** stock and mix by pipetting (do **NOT** vortex). To reactions designated for H3K4me3 & IgG Control Antibodies, add 2 µL **K-MetStat Panel** and vortex to mix. **Note:** If using <500,000 cells, decrease K-MetStat Panel per instructions on p. 16 of the manual.

Section IV: Antibody Binding (~30 min + overnight), continued

17. Add 0.5 µg antibody to each sample. For designated control reactions, add 1µL **IgG** or **H3K4me3 Control Antibody**. Gently vortex to mix.
18. Incubate overnight on a nutator at 4ºC with caps slightly elevated. Do **NOT** rotate tubes.

DAY 2

Section V: Binding of pAG-MNase (~40 min)

19. Place a reagent reservoir on ice. Fill with cold **Cell Perm. Buffer**.
20. Remove tubes from 4˚C, quick spin to collect liquid. **Note:** Beads may settle overnight; this is normal.
21. Place tubes on a magnet and allow slurry to clear. Pipette to remove supernatant.
22. Keep tubes on magnet. Add 200 µL/reaction cold **Cell Perm. Buffer**. Remove supernatant. Repeat one time.
23. Remove tubes from magnet. Add 50 µL/reaction cold **Cell Perm. Buffer** and gently vortex to mix.
    Note: Beads may become clumpy at this stage of the protocol, but can be dispersed by gentle pipetting.
24. Add 2.5 µL/reaction **pAG-MNase**. Gently vortex or pipette to resuspend beads and evenly distribute enzyme.
25. Incubate 10 min at RT.
26. Quick spin tubes, place on magnet, and allow slurry to clear. Remove supernatant.
27. Keep tubes on magnet. Add 200 µL/reaction cold **Cell Perm. Buffer**. Remove supernatant. Repeat one time.
28. Remove tubes from magnet. Add 50 µL/reaction cold **Cell Perm. Buffer**. Gently vortex to mix and disperse clumps by pipetting.

Section VI: Targeted Chromatin Digestion and Release (~3 hrs)

29. Place tubes on ice. Add 1 µL/reaction **100 mM Calcium Chloride**, and gently vortex or pipette to evenly mix.
30. Incubate tubes (caps slightly elevated) on a nutator for 2 hours at 4ºC.
31. Prepare **Stop Master Mix**: per reaction, combine 1 µL ***E. coli* Spike-in DNA** and 33 µL **Stop Buffer.** Gently vortex to mix. **Note:** If using <500,000 cells, dilute *E. coli* Spike-in DNA per instructions in **Appendix 2**.
32. At end of incubation, add 34 µL **Stop Master Mix** to each reaction. Gently vortex to mix.
33. Place reactions in a thermocycler set to 37ºC. Incubate for 10 min.
34. Quick-spin tubes, place on magnet, and allow slurry to clear. Transfer supernatants containing CUT&RUN-enriched DNA to new **8-strip tubes**. Discard tubes with ConA beads.

Section VII: DNA Purification (~30 min)

35. Prepare 85% Ethanol (EtOH) **FRESH** using 100% EtOH and molecular biology grade water.
36. Resuspend **SPRIselect** reagent (manufactured by Beckman Coulter, Inc.*) and slowly add 119 µL/reaction.
37. Gently vortex tubes to mix and quick spin to collect liquid. Incubate 5 min at RT.
38. Place tubes on magnet for 2-5 min. Pipette to remove supernatant, do not disturb beads with pipette tip.
39. Keep tubes on magnet. Add 180 µL/reaction **85% EtOH**. Remove supernatant. Repeat one time.
40. Quick spin tubes with caps facing in, so that beads stay in place on side of tubes. Return tubes to magnet and remove residual EtOH.
41. Remove tubes from magnet, leaving caps open. Air-dry beads 2-3 min at RT or until liquid is evaporated but beads still appear damp matte brown. If beads are crackly/light brown, they are too dry.
42. Add 17 µL/reaction **0.1X TE Buffer** to elute DNA.
43. Vortex to resuspend beads and incubate 2 min at RT.
44. Place tubes on magnet for 2 min. Transfer 15 µL CUT&RUN DNA to new **8-strip tubes**.
45. Use 1 µL to quantify DNA with the Qubit fluorometer. Continue to library prep or store DNA at -20ºC.

    See manual for expected results. Do **NOT** examine CUT&RUN DNA on the TapeStation/Bioanalyzer. DNA yields are too low for detection on these platforms and they **will not provide useful information at this step of the workflow**. Wait until **after library prep** to examine fragment distribution.