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.

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* see note, p. 2	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.
5. Repeat the previous step one time.
6. Resuspend beads in 11 µL/reaction cold **Bead Activation Buffer**.
7. Aliquot 10 µL/reaction of beads into **8-strip tubes**. Place on ice.

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

8. Harvest 500,000 cells/reaction. 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.
10. Repeat the previous step one time.
11. Resuspend cells in 105 µL/reaction RT **Wash Buffer**. Add 100 µL cells to 10 µL **activated ConA beads** in 8-strip tubes. Gently vortex until evenly resuspended and quick spin to collect beads.
12. Incubate 10 min at RT to adsorb cells to beads.
13. Place tubes on a magnet, allow slurry to clear, and pipette to remove supernatant.
14. Remove tubes from magnet. Immediately add 50 µL/reaction cold **Antibody Buffer** and pipette to resuspend.

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

15. 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. If using <500,000 cells, decrease K-MetStat Panel amount per manual instructions.
16. Add 0.5 µg antibody to each sample and gently vortex to mix. For designated control reactions, add 1µL **IgG** or **H3K4me3 Control Antibody**. Vortex to mix.
17. Incubate overnight on a nutator at 4°C, caps elevated. Do **NOT** rotate tubes.

DAY 2

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

18. Quick spin tubes, place on a magnet, and allow slurry to clear. Pipette to remove supernatant.
19. Keeping tubes on the magnet, add 200 µL/reaction cold **Cell Perm. Buffer**. Remove supernatant.
20. Repeat the previous step one time, keeping tubes on the magnet.
21. Remove tubes from magnet. Add 50 µL/reaction cold **Cell Perm. Buffer** and gently vortex or pipette to mix.
22. Add 2.5 µL/reaction **pAG-MNase**. Gently vortex or pipette to mix. Ensure beads are fully resuspended.
23. Incubate 10 min at RT. Return tubes to magnet and allow slurry to clear. Remove supernatant.
24. Keeping tubes on the magnet, add 200 µL cold **Cell Perm. Buffer**. Remove supernatant.
25. Repeat the previous step one, time keeping tubes on the magnet.
26. Remove tubes from magnet. Add 50 µL/reaction cold **Cell Perm. Buffer** and gently vortex or pipette to mix.

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

27. Place tubes on ice. Add 1 µL/reaction **100 mM Calcium Chloride**, and gently vortex or pipette to evenly mix.
28. Incubate tubes (caps slightly elevated) on nutator for 2 hours at 4°C.
29. During incubation, prepare a **Stop Master Mix**: per CUT&RUN reaction, combine 1 µL ***E. coli* Spike-in DNA*** and 33 µL **Stop Buffer.** Gently vortex or pipette to mix.
30. At end of incubation, add 33 µL **Stop Master Mix** to each reaction. Gently vortex to mix.
31. Place reactions in a thermocycler set to 37°C. Incubate for 10 min.
32. Quick-spin tubes to collect liquid, place on magnet, and allow slurry to clear. Transfer supernatants to fresh 1.5 mL tubes and discard beads.

Section VII: DNA Purification (~30 min)

Prior to first use:

Add 6.9 mL isopropanol to **DNA Binding Buffer**. Add 20 mL ≥95% Ethanol to **DNA Wash Buffer**.

33. Add 420 µL **DNA Binding Buffer** to each reaction and vortex.
34. Load each reaction into a labeled **DNA Cleanup Column** + **Collection Tube**.
35. Spin at 16,000 x g for 30 sec at RT. Discard flow-through.
36. Add 200 µL **DNA Wash Buffer**. Spin at 16,000 x g, 30 sec, RT. Discard flow-through.
37. Repeat the previous step one time.
38. Spin one additional time at 16,000 x g, 30 sec, RT.
39. Transfer column to a clean 1.5 mL tube. Add 12 µL **DNA Elution Buffer** directly to the center of the column.
40. Let sit for 5 min, then spin at 16,000 x g, 1 min, RT to elute DNA.
41. Vortex and use 1 µL to quantify CUT&RUN DNA using the Qubit™ fluorometer. DNA can be stored at -20°C.

Section VIII-X: Library Prep and Illumina® Sequencing *(see manual for full details)*

42. Prepare paired-end sequencing libraries from CUT&RUN enriched DNA (~5 ng preferred, but can use less). We recommend the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001 & 14-1002).
43. Confirm enrichment of mononucleosomal fragments (~300 bp, including sequencing adapters) and library concentration by electrophoretic mobility analysis (e.g. Agilent Bioanalyzer® or TapeStation®).
44. Perform Illumina® sequencing. Aim for 3-8 million uniquely aligned reads per reaction.

***NOTE:** The amount of **Digitonin** and ***E. coli* Spike-in DNA** should be optimized for the cell type and target(s) of interest. See manual for full details.