Catalog No. 14-1048 and 14-1048-24

CUTANA™ ChIC / CUT&RUN Kit Version 5

QUICK-START CARD

SCAN FOR MANUAL
Read latest manual before first experiment!

DAY 1 (~2 HOURS)

1. Prepare buffers as outlined below. Recipes contain 20% excess - no overage is needed. Use Digitonin concentrations as optimized for each cell type (protocol at support.epicypher.com).

BUFFER	COMPONENTS	1X	8X	16X	STORAGE
Wash Buffer	Pre-Wash Buffer	1.8 mL	14.4 mL	28.8 mL	Room temp (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 Perm. 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	

2. Resuspend **ConA Bead** stock and transfer 11 µL/reaction to a 1.5 mL tube. Place tube on a compatible magnetic rack, allow slurry to clear, and pipette to remove supernatant.
3. Take tube off magnet and resuspend beads in 100 µL/reaction cold **Bead Activation Buffer**. Place on magnet, allow slurry to clear, and remove supernatant. Repeat one time.
4. Resuspend beads in 11 µL/reaction cold **Bead Activation Buffer**. Place on ice.
5. Collect cells and spin at 600 x g for 3 min at RT. Remove supernatant and resuspend in 1 mL 1X PBS. Take a 10 µL aliquot of cells, add 10 µL 0.4% Trypan Blue, and mix. Transfer to a cell counting slide. Obtain cell counts, determine viability (>80%), and confirm cell integrity.
6. Harvest 500,000 cells/reaction plus 10% excess. Spin 600 x g, 3 min, RT. Remove supernatant.
7. Resuspend in 100 µL/reaction RT **Wash Buffer**. Spin 600 x g, 3 min, RT. Remove supernatant. Repeat one time.
8. Resuspend cells in 105 µL/reaction RT **Wash Buffer**. Take 10 µL cells and perform Trypan Blue staining as in Step 5. Obtain cell counts and confirm cell integrity.
9. Add 10 µL/reaction **activated ConA Beads** to washed cells. Gently vortex to resuspend and quick spin in a mini-centrifuge to collect liquid. Incubate 10 min at RT to adsorb cells to beads.
10. Place tube on a magnet, allow slurry to clear. Note that supernatant should not contain cells. To confirm, take 10 µL supernatant and perform Trypan Blue staining as in Step 5.
11. Discard remaining supernatant. Resuspend slurry in 55 µL/reaction cold **Antibody Buffer**.
12. Confirm cells are permeabilized and bound to ConA beads: take 10 µL slurry and perform Trypan Blue staining as in Step 5. See kit manual for examples and further details.
13. Aliquot 50 µL/reaction bead slurry to **8-strip Tubes**.
14. Quick spin the **K-MetStat Panel** stock and mix by pipetting (do **NOT** vortex stock). Add **K-MetStat Panel** to reactions designated for H3K4me3, H3K27me3, and IgG Control Antibodies. Add 2 µL if using 500,000 cells/reaction. For lower cell numbers, decrease K-MetStat Panel per manual instructions. Gently vortex tubes to mix and quick spin.
15. Add 0.5 µg primary antibody to each reaction. For control reactions, add 1 µL of respective **H3K4me3, H3K27me3, or IgG Control Antibody**. Gently vortex and quick spin.
16. Incubate overnight on a nutator at 4°C, gently rocking tubes on a nutator with caps elevated. Do **NOT** rotate tubes end-over-end, as this will result in sample loss.

DAY 2 (~7 HOURS)

17. If using a multi-channel pipettor, place a reagent reservoir on ice. Fill with cold **Cell Perm. Buffer**. Always remove and replace buffers one tube strip at a time to avoid bead dry out.
18. Quick spin reaction tubes to collect liquid. Place tubes on a compatible 8-strip tube magnet and allow slurry to clear. Pipette to remove supernatant.
19. On magnet, add 200 µL/reaction cold **Cell Perm. Buffer**. Remove supernatant. Repeat one time.
20. Take tubes off magnet and resuspend beads in 50 µL/reaction cold **Cell Perm. Buffer**. Disperse bead clumps by gentle pipetting and/or vortexing. Quick spin tubes to collect liquid.
21. Add 2.5 µL/reaction **pAG-MNase**. Gently vortex or pipette to mix beads and distribute enzyme.
22. Quick spin tubes and incubate 10 min at RT.
23. Quick spin tubes, place on magnet, and allow slurry to clear. Remove supernatant.
24. On magnet, add 200 µL/reaction cold **Cell Perm. Buffer**. Remove supernatant. Repeat one time.
25. Take tubes off magnet and resuspend in 50 µL/reaction cold **Cell Perm. Buffer**. Gently vortex to mix and disperse clumps by pipetting. Quick spin tubes and place on ice.
26. Add 1 µL/reaction **100 mM Calcium Chloride**. Gently vortex or pipette to evenly resuspend.
27. Quick spin tubes. Incubate on a nutator for 2 hours at 4°C, caps slightly elevated. Do not rotate!
28. Prepare **Stop Master Mix** in a new 1.5 mL tube. 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/reaction, reduce the amount of *E. coli* DNA. Visit support.epicypher.com for guidance.
29. At end of incubation, add 33 µL/reaction **Stop Master Mix**. Gently vortex to mix and quick spin.
30. Place reactions in a thermocycler set to 37°C. Incubate for 10 min.
31. Quick spin tubes, place on magnet, and allow slurry to clear. Transfer supernatants containing CUT&RUN-enriched chromatin to new **8-strip Tubes**. Discard tubes with ConA beads.
32. Prepare 500 µL 85% Ethanol (EtOH) per reaction by combining 425 µL 100% EtOH and 75 µL molecular biology grade water. Prepare fresh, mix well, and store at RT.
33. Vortex **SPRI beads** to thoroughly resuspend beads. Slowly add 118 µL/reaction.
34. Mix well by pipetting and/or vortexing. Quick spin to collect liquid. Incubate 5 min at RT.
35. Place tubes on magnet for 2-5 min. Pipette to remove supernatant without disturbing beads.
36. On magnet, add 180 µL/reaction **85% EtOH**. Remove supernatant. Repeat one time.
37. Quick spin with caps facing in to avoid dislodging beads. Place on magnet and remove residual EtOH.
38. Take tubes off magnet. Air-dry, caps open, 2-3 min at RT. Beads should be damp matte brown (Figure 1). If beads are crackly/light brown, they are too dry.
39. Add 17 µL/reaction **0.1X TE Buffer** to elute DNA. Pipette or vortex to resuspend beads and quick spin.
40. Incubate 2 min at RT.

FIGURE 1
Elute DNA when "ideal."

41. Quick spin tubes and place on magnet for 2 min. Transfer 15 µL CUT&RUN-enriched DNA DNA to new **8-strip Tubes**.
42. Use 1 µL to quantify DNA with the Qubit fluorometer. Proceed to library prep using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001 / 14-1002) or store DNA at -20°C.

    Do **NOT** examine CUT&RUN DNA on the TapeStation/Bioanalyzer, as DNA yields are too low. Wait until **after library prep** to examine fragment distribution.