Kit v2.0

CUTANA™ ChIC/CUT&RUN Kit

QUICK-START CARD

Scan for full manual

DAY 1

Section I: CUTANA Spike-in Controls & Buffer Preparation (~1 hr)

1. In 1.5 mL tube, pipette mix 4 µL **SA Beads** with 150 µL **Pre-Wash Buffer**. Place on magnet and remove supernatant (sup).
2. Remove from magnet and resuspend in 210 µL **Pre-Wash Buffer**. Aliquot 50 µL bead slurry into 4 x 1.5 mL tubes.
3. Add 1 µL **CUTANA Spike-in Control Unmodifed dNuc** to a 1.5 mL tube and pipette mix. Repeat for remaining **CUTANA Spike-in Control dNucs** (H3K4me1, H3K4me2 and H3K4me3), adding each dNuc into a separate tube.
4. Incubate 30 min at RT on nutator. During incubation, prepare buffers:

BUFFER NAME	COMPONENTS	1X	8X	16X	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	

5. Quick spin **CUTANA Spike-in Control** bead slurries to collect samples. Place on magnet and remove sup.
6. Remove tubes from magnet. Add 150 µL **Pre-Wash Buffer**. Place back on magnet and remove sup.
7. Remove tubes from magnet and resuspend beads in 5 µL **Pre-Wash Buffer**. Combine all 4 dNucs into a single tube (20 µL).
8. Place back on magnet and remove sup. Remove from magnet and resuspend in 8 µL **Antibody Buffer**. Place immobilized **CUTANA H3K4 MetStat Spike-in Controls** on ice until needed.

Section II: Bead Activation (~30 min)

9. Gently resuspend **ConA Beads** and transfer 11 µL/sample to 1.5 mL tube.
10. Place tube on magnet until slurry clears and remove sup.
11. Add 100 µL/sample cold **Bead Activation (BA) Buffer** and mix. Place the tube on magnet until slurry clears and remove sup.
12. Repeat previous step for total of two washes.
13. Resuspend beads in 11 µL/sample cold **BA Buffer** and aliquot 10 µL/sample of bead slurry into **8-strip tubes**. Keep on ice.

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

14. Harvest 0.5 million cells/sample in 1.5 mL tube. Centrifuge 3 min at 600 x g at room temperature (RT). Remove sup.
15. Add 100 µL/sample RT **Wash Buffer**. Resuspend cells by thorough pipetting. Centrifuge 3 min at 600 x g, RT. Remove sup.
16. Repeat previous step for total of two washes.
17. Resuspend cells in 105 µL/sample in RT **Wash Buffer**. Aliquot 100 µL washed cells to each **8-strip tube** containing 10 µL activated beads.
18. Gently vortex to mix, then incubate on benchtop for 10 min at RT.

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

19. Place tubes on a magnet until slurry clears and remove sup. Add 50 µL/sample cold **Antibody Buffer**, gently vortex.
20. Add 2 µL immobilized **CUTANA H3K4 MetStat Spike-in Controls** to the samples designated for **H3K4me3** and **IgG Control Antibodies**.
21. Add 0.5 µg antibody (controls + targets of interest) to each sample and gently vortex.
22. Incubate **8-strip tubes** (caps slightly elevated) on nutator overnight at 4°C.

DAY 2

23. Place the **8-strip tubes** on a magnet until slurry clears and pipette to remove sup.
24. With beads on the magnet, add 200 µL cold **Cell Permeabilization Buffer** directly onto beads. Pipette to remove sup.
25. Repeat previous step for total of two washes without removing beads from magnet.
26. Remove beads from magnet. Add 50 µL/sample cold **Cell Permeabilization Buffer**. Thoroughly pipette/vortex to mix.

Section V: Binding of pAG-MNase (~30 min)

27. Ensure beads are completely resuspended. Add 2.5 µL/sample **pAG-MNase** (20x stock), and gently vortex.
28. Incubate samples for 10 min at RT. Return **8-strip tube** to magnet and remove sup.
29. While beads are on magnet, add 200 µL/sample cold **Cell Permeabilization Buffer** directly onto beads. Remove sup.
30. Repeat previous step for total of two washes without removing beads from magnet.
31. Remove beads from magnet. Add 50 µL/sample cold **Cell Permeabilization Buffer**. Thoroughly pipette/vortex to mix.

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

32. Place **8-strip tubes** on ice. Add 1 µL/sample **100 mM Calcium Chloride**, and gently vortex.
33. Incubate **8-strip tubes** on nutator for 2 hours at 4°C.
34. Add 33 µL/sample **Stop Buffer**, and gently vortex to mix.
35. Add 1 µL ***E. coli* Spike-in DNA***, and gently vortex to mix. Incubate **8-strip tubes** for 10 min at 37°C in thermocycler.
36. Quick-spin in microfuge. Place **8-strip tubes** on magnet until slurry clears. Transfer sups to fresh 1.5 mL tubes and discard beads.

Section VII: DNA Purification (~30 min)

37. Add 420 µL/sample **DNA Binding Buffer** and vortex. Load onto a **DNA Cleanup Column** + **Collection Tube**.
38. Centrifuge for 30 sec at 16,000 x g, RT. Discard flow-through.
39. Add 200 µL **DNA Wash Buffer**. Centrifuge for 30 sec at 16,000 x g, RT. Discard flow-through.
40. Repeat for total of two washes. Discard flow-through and centrifuge one additional time.
41. Transfer column to a clean 1.5 mL tube. Add 12 µL **DNA Elution Buffer** directly to the column center. Let sit 5 minutes, then centrifuge 1 min at 16,000 x g, RT.
42. Vortex and use 1 µL to quantify CUT&RUN DNA using the Qubit™ fluorometer. DNA can be stored at -20°C.

Section VIII: Library Preparation and NGS *(see manual for full details)*

43. Prepare paired-end Illumina sequencing libraries from CUT&RUN enriched DNA (5-10 ng preferred, but can use less).
44. Confirm enrichment of mononucleosomal fragments (~150 bp + sequencing adapters) by electrophoretic mobility analysis (e.g. 10 ng DNA analyzed by Agilent Bioanalyzer or equivalent).
45. Perform Illumina sequencing, aiming for 3-8 million reads/sample.

***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.*