CUTANA™ CUT&TAG Kit

QUICK-START CARD

Scan for full manual - read before first use

DAY 1

Section I: Buffer Prep (~30 min)

1. Prepare buffers as outlined below. Recipes contain 20% excess - no additional overage is needed.

BUFFER	COMPONENTS	1X	8X	16X	STORAGE
Nuclear Extraction Buffer	Pre-Nuclear Extract Buffer	235 µL	1.9 mL	3.8 mL	Ice for use on Day 1
	25X Protease Inhibitor	9.8 µL	78.4 µL	157 µL	
	1 M Spermidine	0.13 µL	1.0 µL	2.0 µL	
Wash Buffer 1	Pre-Wash Buffer	1.3 mL	10.4 mL	20.8 mL	4°C for use on Day 2
	25X Protease Inhibitor	56 µL	448 µL	896 µL	
	1 M Spermidine	0.7 µL	5.6 µL	11.2 µL	
	5% Digitonin	2.8 µL	22.4 µL	44.8 µL	
Wash Buffer 2	Wash Buffer 1	600 µL	4.8 mL	9.6 mL	4°C for use on Day 2
	4.5 M NaCl	20.7 µL	166 µL	331 µL	
Antibody Buffer	Wash Buffer 1	60 µL	480 µL	960 µL	Ice for use on Day 1
	0.5 M EDTA	0.25 µL	2 µL	4 µL	

Section II: Bead Activation (~30 min)

2. Resuspend **ConA beads** and transfer 11 µL/reaction to a 1.5 mL tube. Place tube on a magnetic rack, allow slurry to clear, and pipette to remove supernatant.
3. Resuspend beads in 100 µL/reaction cold **Bead Activation Buffer**. Return to magnet, allow slurry to clear, and remove supernatant. Repeat one time.
4. Resuspend beads in 11 µL/reaction cold **Bead Activation Buffer**. Aliquot 10 µL/reaction activated ConA beads into **8-strip tubes**. Keep on ice.

Section III: Nuclei Prep and Binding to Beads (~30 min)

5. Harvest 100,000 cells/reaction in a 1.5 mL tube. Spin 600 x g for 3 min at room temperature (RT).
6. Remove supernatant and resuspend cells in 100 µL/reaction cold **Nuclear Extraction Buffer**.
7. Incubate 10 min on ice. Spin 600 x g for 3 min at 4˚C. Pipette to remove supernatant.
8. Resuspend nuclei in 105 µL/reaction cold **Nuclear Extraction Buffer**. Add 100 µL nuclei to 10 µL ConA beads in 8-strip tubes. Gently vortex to mix and quick spin to collect liquid.
9. Incubate 10 min at RT. Place tubes on magnet, allow slurry to clear, and remove supernatant.
10. Resuspend in 50 µL/reaction cold **Antibody Buffer**.

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

11. Quick spin the **K-MetStat Panel** tube and mix by pipetting (do **NOT** vortex stock). To reactions designated for H3K27me3 and IgG control antibodies, add 2 µL **K-MetStat Panel** and vortex to mix. **Note:** If using <100,000 nuclei, decrease K-MetStat Panel amount as per the manual instructions.
12. Add 0.5 µg primary antibody to each reaction. For designated control reactions, add 1µL **H3K27me3 Positive Control Antibody** and 1 µL **IgG Negative Control Antibody**. Vortex to mix and quick spin.
13. Incubate overnight on a nutator at 4°C, gently rocking tubes with caps elevated. Do **NOT** rotate tubes end-over-end, as this will result in sample loss.

DAY 2

Section V: Secondary Antibody Binding (~1 hr)

14. Prepare **Tagmentation Buffer** in a new 1.5 mL tube. Per reaction, combine 59.4 µL **Wash Buffer 2** and 0.6 µL **1M $MgCl_2$** (10 mM final concentration). Place on ice. Recipe includes 20% excess volume.
15. Quick spin tubes, place on a magnet, and allow slurry to clear. Remove supernatant and resuspend in 50 µL/reaction cold **Wash Buffer 1** by pipetting and/or vortexing. Quick spin to avoid bead loss. ***If pipetting:** Carefully mix, avoid bead loss in tip, and expel all material back into tubes.
16. Add 0.5 µg/reaction secondary antibody. Use 0.5 µL **Anti-Rabbit Secondary Antibody** for control & rabbit primary antibodies. Gently vortex and quick spin. Place on nutator, caps elevated, 30 min RT.
17. Gently vortex tubes, quick spin. Place tubes on magnet, allow slurry to clear, and remove supernatant.
18. On magnet, add 200 µL/reaction cold **Wash Buffer 1**. Remove supernatant. Repeat one time.
19. Resuspend in 50 µL/reaction cold **Wash Buffer 2** by pipetting and/or vortexing (see ***** in Step 15).

Section VI: pAG-Tn5 Binding & Targeted Chromatin Tagmentation (~4 hrs)

20. Add 2.5 µL/reaction **pAG-Tn5**. Gently vortex and quick spin. Place on nutator, caps elevated, 1 hr RT.
21. Gently vortex and quick spin. Place tubes on a magnet, allow slurry to clear, and remove supernatant.
22. Resuspend in 200 µL/reaction cold **Wash Buffer 2** by pipetting (see ***** in Step 15). Quick spin tubes and return to magnet. Allow slurry to clear, remove supernatant. Repeat one time.
23. Resuspend in 50 µL/reaction cold **Tagmentation Buffer** by pipetting (see ***** in Step 15).
24. Gently vortex tubes ~5 sec and quick spin. Incubate 1 hr in thermocycler set to 37˚C (lid at 47˚C). For step 26: transfer 60 µL/reaction **Pre-Wash Buffer** to a new tube and equilibrate to RT.
25. Gently vortex and quick spin. Place tubes on magnet, allow slurry to clear, and remove supernatant.
26. Add 50 µL/reaction RT **Pre-Wash Buffer** and pipette 3-5 times to resuspend beads (expel all material back into tubes). Do **NOT** vortex. Place tubes on magnet, allow slurry to clear. Remove supernatant.
27. Add 5 µL/reaction RT **SDS Release Buffer**. Do **NOT** pipette. Vortex ~10 sec at max speed to mix and quick spin tubes. Incubate 1 hr in a thermocycler set to 58˚C (lid at 68˚C).
28. Quick spin tubes. Add 15 µL/reaction RT **SDS Quench Buffer**, carefully pipetting to rinse beads (skip pipetting if too viscous). Vortex ~10 sec at max speed, quick spin tubes, and keep at RT.

Section VII: Indexing PCR & Library Cleanup (~1 hr)

29. Assign a unique pair of **i5 & i7 indexing primers** to each reaction (**Appendix 3**). To the entire reaction mixture add: 2 µL **i5 primer**, 2 µL **i7 primer**, and 25 µL **Non-Hot Start 2X PCR Master Mix**. Mix well, avoid bubbles, and quick spin.
30. Perform PCR using parameters in the table (lid at 105˚C). During PCR, prepare 500 µL/reaction **85% EtOH**.
31. Quick spin tubes. Fully resuspend **SPRIselect reagent** (manufactured by Beckman Coulter, Inc.) and slowly add 65 µL/reaction. Mix well by vortexing/pipetting.
32. Quick spin and incubate 5 min at RT. Place tubes on magnet for 2-5 min. Pipette to remove supernatant.

STEP #	TEMP	TIME	CYCLES
1	58˚C	5 min	1
2	72˚C	5 min	1
3	98˚C	45 sec	1
4	98˚C	15 sec	14-21
5	60˚C	10 sec	
6	72˚C	1 min	1
7	4-12˚C	∞	1

33. On magnet, add 180 µL/reaction **85% EtOH**. Pipette to remove supernatant. Repeat one time.
34. Quick spin tubes with caps facing in. Return to magnet and pipette to remove residual EtOH.
35. Take tubes off magnet. Air-dry, caps open, for 2-3 min at RT. Beads should be damp matte brown.
36. Add 17 µL/reaction **0.1X TE Buffer**. Pipette/vortex to resuspend beads. Incubate 2 min at RT.
37. Quick spin tubes, place on magnet for 2 min. Transfer 15 µL CUT&Tag libraries to new **8-strip tubes**.

Section VIII: Analysis of Library Fragment Size (~1 hr)

38. Quantify libraries using the Qubit™ fluorometer. Examine fragment size distribution on the Agilent TapeStation® or Bioanalyzer®. Proceed to sequencing or store at -20ºC.