CUTANA™ CUT&TAG Kit

QUICK-START CARD

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DAY 1

Section I: Buffer Prep (~30 min)

1. Prepare CUT&Tag buffers (see Table). Buffers contain 20% excess volume - no additional overage is needed.

BUFFER	COMPONENTS	1X	8X	16X	STORAGE
Nuclear Extraction Buffer	Pre-Nuclear Extraction 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 magnet, 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**.
5. Aliquot 10 µL/reaction of bead slurry into **8-strip tubes**. Place on ice.

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

6. Harvest 100,000 cells/reaction in a 1.5 mL tube. Spin 600 x g for 3 min at room temperature (RT).
7. Remove supernatant and resuspend cells in 100 µL/reaction cold **Nuclear Extraction Buffer**.
8. Incubate 10 min on ice. Spin 600 x g for 3 min at 4˚C. Pipette to remove supernatant.
9. 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 bead slurry.
10. Incubate 10 min at RT to adsorb cells to beads.
11. Place tubes on magnet, allow slurry to clear, remove supernatant. Take tubes off magnet and resuspend in 50 µL/reaction cold **Antibody Buffer**.

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

12. Quick spin the **K-MetStat Panel** stock and mix by pipetting (do **NOT** vortex). To reactions designated for H3K27me3 & IgG control antibodies, add 2 µL **K-MetStat Panel** and vortex to mix. If using <100,000 nuclei, decrease K-MetStat Panel amount as per the manual instructions.
13. Add 0.5 µg primary antibody to each reaction. For designated control reactions, add 1µL **H3K27me3 positive control antibody** & 1 µL **IgG negative control antibody**. Vortex to mix.
14. Incubate overnight on a nutator at 4°C, caps elevated. Do **NOT** rotate tubes.

DAY 2

Section V: Secondary Antibody Binding (~1 hr)

15. Prepare **Tagmentation Buffer**: Transfer 60 µL/reaction **Wash Buffer 2** to a 1.5 mL tube. Add 1 M $MgCl_2$ at a 1:100 dilution (10 mM final concentration). Place on ice. Recipe includes 20% excess volume.
16. Quick spin tubes, place on a magnet, and allow slurry to clear. Remove supernatant and resuspend in 50 µL/reaction cold **Wash Buffer 1**.
17. Add 0.5 µg/reaction secondary antibody. For control antibodies and other rabbit primary antibodies, use 0.5 µL **anti-rabbit secondary antibody**. Gently vortex to mix.
18. Incubate on nutator, caps elevated, for 30 min at RT.
19. Quick spin tubes, place on a magnet, and allow slurry to clear. Remove supernatant.
20. On magnet, add 200 µL/reaction cold **Wash Buffer 1**. Remove supernatant. Repeat one time.
21. Remove tubes from magnet and resuspend in 50 µL/reaction cold **Wash Buffer 2**.

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

22. Add 2.5 µL/reaction **pAG-Tn5** and gently vortex to mix. Incubate on nutator, caps elevated, for 1 hr at RT.
23. Quick spin tubes, place on a magnet, and allow slurry to clear. Remove supernatant.
24. Resuspend in 200 µL/reaction cold **Wash Buffer 2**. Return tubes to magnet, allow slurry to clear, and remove supernatant. Repeat one time.
25. Resuspend in 50 µL/reaction cold **Tagmentation Buffer**. Incubate 1 hr in a thermocycler with block set to 37˚C and heated lid set to 47˚C.
26. Quick spin tubes, place on magnet, and allow slurry to clear. Remove supernatant.
27. Add 50 µL/reaction **TAPS Buffer** and gently vortex to resuspend (do **NOT** pipette-mix after adding TAPS Buffer). Place on magnet, allow slurry to clear, and remove supernatant.
28. Add 5 µL/reaction **SDS Release Buffer**, vortex to mix (do **NOT** pipette), and quick spin.
29. Incubate 1 hr in a thermocycler with block set to 58˚C and heated lid set to 68˚C.
30. Quick spin tubes. Add 15 µL/reaction **SDS Quench Buffer**, vortex to mix (do **NOT** pipette), and quick spin.

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

31. Assign a unique pair of **i5 & i7 indexing primers** to each reaction. See kit manual, **Appendix 3**.
32. To the entire reaction mixture (including ConA beads) add: 2 µL **i5 primer**, 2 µL **i7 primer**, and 25 µL **Non-Hot Start 2X PCR Master Mix**. Vortex to mix and quick spin to collect liquid.
33. Perform PCR in a thermocycler with a heated lid (105˚C) using the parameters in the table. During PCR, prepare **80% EtOH**.
34. After PCR, quick spin tubes. Resuspend **SPRIselect** reagent (manufactured by Beckman Coulter, Inc.*) and slowly add 65 µL/reaction. Vortex to mix and quick spin.

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˚C	∞	1

35. Incubate 5 min at RT. Place tubes on magnet for 2 min. Pipette to remove supernatant.
36. On magnet, add 200 µL/reaction **80% EtOH**. Pipette to remove supernatant. Repeat one time.
37. Quick spin and return tubes to magnet. Remove residual EtOH.
38. Remove tubes from magnet and air-dry 2 min at RT, caps open.
39. Add 17 µL/reaction **0.1X TE Buffer** to elute libraries. Vortex to resuspend beads and incubate 2 min at RT.
40. Place tubes on magnet for 2 min. Transfer 15 µL eluted CUT&Tag libraries to new **8-strip tubes**.

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

41. Quantify libraries using the Qubit™ fluorometer and 2X dsDNA HS Assay Kit.
42. Confirm fragment size distribution (~300 bp) on the Agilent BioAnalyzer® or TapeStation®.
43. Store prepared libraries at -20ºC. See manual for guidance on sample pooling and Illumina® sequencing.