CUTANA™ CUT&TAG Kit

QUICK-START CARD

Scan for full manual - read before first use

DAY 1 (~2 HOURS)

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

BUFFER	COMPONENTS	1X	8X	16X	STORAGE
Nuclei Extraction Buffer	Pre-Nuclei 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	

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.
5. Harvest 100,000 cells/reaction. Spin 600 x g, 3 min, room temperature (RT). **High-quality sample prep is crucial - see kit manual or support.epicypher.com for guidance!**
6. Remove supernatant and resuspend cells in 100 µL/reaction cold **Nuclei Extraction Buffer**.
7. Incubate 10 min on ice. Spin 600 x g for 5 min at 4˚C. Pipette to remove supernatant.
8. Resuspend nuclei in 105 µL/reaction cold **Nuclei 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**.
11. Quick spin the **K-MetStat Panel** tube 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 100,000 nuclei/reaction. For lower nuclei numbers, decrease K-MetStat Panel per manual instructions. Gently vortex tubes to mix and quick spin.
12. Add 0.5 µg primary antibody to each reaction. For control reactions, add 1 µL of respective **H3K27me3, H3K4me3, or IgG Control Antibody**. Gently vortex and quick spin.
13. Incubate overnight on a nutator at 4°C, gently rocking tubes with caps elevated. Do **NOT** rotate tubes, as this will result in sample loss.

DAY 2 (~7 HOURS)

Mixing, steps 17-25: Carefully pipette, avoid bead loss in tip, expel all material back into tubes.

Before and after all incubation steps: Gently vortex tubes ~5 sec and quick spin to collect liquid.

14. Prepare **Tagmentation Buffer** in a new 1.5 mL tube. Per reaction, combine 59.4 µL **Wash Buffer 2** and 0.6 µL **1 M $MgCl_2$** (10 mM final). Place on ice. Recipe includes 20% excess.
15. For step 25, transfer 60 µL/reaction **Pre-Wash Buffer** to a new tube and place at RT.
16. Quick spin reaction tubes, place on a magnet, and allow slurry to clear. Remove supernatant.
17. Resuspend in 50 µL/reaction cold **Wash Buffer 1**. Add 0.5 µg/reaction secondary antibody. Use 0.5 µL **Anti-Rabbit Secondary Antibody** for control and rabbit primary antibodies.
18. Incubate 30 min on a nutator at RT. Place on magnet, allow slurry to clear, remove supernatant.
19. On magnet, add 200 µL/reaction cold **Wash Buffer 1**. Remove supernatant. Repeat one time.
20. Resuspend in 50 µL/reaction cold **Wash Buffer 2**. Add 2.5 µL/reaction **pAG-Tn5**.
21. Incubate 1 hr on a nutator at RT. Place on magnet, allow slurry to clear, remove supernatant.
22. Resuspend in 200 µL/reaction cold **Wash Buffer 2**. Place on a magnet, allow slurry to clear, remove supernatant. Repeat one time.
23. Resuspend in 50 µL/reaction cold **Tagmentation Buffer**.
24. Incubate 1 hr in thermocycler set to 37˚C (lid at 47˚C). Place on magnet, allow slurry to clear, remove supernatant.
25. Resuspend in 50 µL/reaction RT **Pre-Wash Buffer**. Do **NOT** vortex. Place tubes on magnet, allow slurry to clear. Remove supernatant.
26. Add 5 µL/reaction RT **SDS Release Buffer**. Do **NOT** pipette. Vortex ~10 sec and quick spin.
27. Incubate 1 hr in a thermocycler set to 58˚C (lid at 68˚C).
28. Add 15 µL/reaction RT **SDS Quench Buffer**, carefully pipetting to rinse beads (vortex if viscous). Vortex ~10 sec max speed to mix, quick spin, and keep at RT.
29. Assign a unique pair of **i5 & i7 Primers** per reaction (**Appendix 1**). To the entire reaction 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.

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

30. Perform PCR per table parameters (lid at 105˚C). During PCR, prep 500 µL/reaction **85% EtOH**.
31. Quick spin tubes. Vortex **SPRIselect reagent** (manufactured by Beckman Coulter, Inc.) to fully resuspend and slowly add 65 µL/reaction. Mix well by vortexing and/or pipetting.
32. Incubate 5 min at RT. Place tubes on magnet for 2-5 min. Remove supernatant.
33. On magnet, add 180 µL/reaction **85% EtOH**. 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, 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 libraries to new **8-strip Tubes**.
38. Quantify libraries and examine fragment size distribution per manual instructions. Proceed to sequencing or store at -20°C.