Catalog No. 14-1102 & 14-1103

CUTANA™ CUT&TAG Kit Version 3

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.

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 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. Determine cell count, viability (>80%) and cell integrity using Trypan Blue staining, outlined in manual. Harvest 100,000 cells/reaction plus 20% excess. Spin 600 x g, 3 min, room temp (RT).
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**. Confirm nuclei extraction using Trypan Blue staining, as outlined in manual.
9. Add 10 µL/reaction **activated ConA Beads** to nuclei. Gently vortex to resuspend and quick spin.
10. Incubate 10 min at RT. Place tubes on magnet, allow slurry to clear. Note: supernatant should not contain nuclei. Confirm by Trypan Blue staining (see manual).
11. Resuspend slurry in 55 µL/reaction cold **Antibody Buffer**. Confirm nuclei bead binding using Trypan Blue staining, as outlined in manual.
12. Aliquot 50 µL/reaction bead slurry to **8-strip Tubes**.
13. Quick spin **K-MetStat Panel** and mix by pipetting (do **NOT** vortex). Add **K-MetStat Panel** to control reactions. Add 2 µL if using 100,000 nuclei/reaction. For lower inputs, see manual.
14. Gently vortex tubes and quick spin. Add 0.5 µg primary antibody to each reaction. For control reactions, add 1 µL of respective **H3K27me3, H3K4me3, or IgG Control Antibody**.
15. Gently vortex and quick spin. Incubate overnight on a nutator at 4ºC, gently rocking tubes with caps elevated. Do **NOT** rotate tubes, as this will result in sample loss.

DAY 2 (~7 HOURS)

16. 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.

Mixing in Steps 17-26: Carefully pipette the slurry, avoiding bead loss in tips, and expel all material back into tubes. Vortexing can also help resuspend beads. **Always** quick spin tubes after mixing.

17. Quick spin reaction tubes, place on a magnet, and allow slurry to clear. Remove supernatant.
18. 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.
19. Gently vortex ~5 sec to mix and quick spin. Incubate 30 min on a nutator at RT, caps elevated.
20. Gently vortex ~5 sec, quick spin. Place on magnet, allow slurry to clear, remove supernatant.
21. On magnet, add 200 µL/reaction cold **Wash Buffer 1**. Remove supernatant. Repeat one time.
22. Resuspend in 50 µL/reaction cold **Wash Buffer 2**. Add 2.5 µL/reaction **pAG-Tn5**.
23. Gently vortex ~5 sec to mix and quick spin. Incubate 1 hour on a nutator at RT, caps elevated.
24. Gently vortex ~5 sec, quick spin. Place on magnet, allow slurry to clear, remove supernatant.
25. Resuspend in 200 µL/reaction cold **Wash Buffer 2**. Quick spin tubes, place on a magnet, allow slurry to clear, and remove supernatant. Repeat one time for a total of two washes.
26. Resuspend in 50 µL/reaction cold **Tagmentation Buffer**. Gently vortex ~5 sec to mix and quick spin. Incubate 1 hr in thermocycler set to 37˚C (lid at 47˚C). During incubation, transfer 60 µL/reaction **Pre-Wash Buffer** to a new tube and place at RT.
27. Gently vortex ~5 sec, quick spin. Place on magnet, allow slurry to clear, remove supernatant.
28. Resuspend in 50 µL/reaction RT **Pre-Wash Buffer**. Do **NOT** vortex. Place tubes on magnet, allow slurry to clear. Remove supernatant.
29. Add 5 µL/reaction RT **SDS Release Buffer**. Do **NOT** pipette. Vortex ~10 sec and quick spin.
30. Incubate 1 hr in a thermocycler set to 58˚C (lid at 68˚C).
31. 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.
32. 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

33. Perform PCR per table parameters (lid at 105˚C). During PCR, prep 500 µL/reaction **85% EtOH**.
34. Quick spin tubes. Vortex **SPRI beads** to fully resuspend. Slowly add 65 µL/reaction.
35. Mix well by vortexing and/or pipetting. Quick spin to collect liquid. Incubate 5 min at RT.
36. Place tubes on magnet for 2-5 min. Pipette to remove supernatant without disturbing beads.
37. On magnet, add 180 µL/reaction **85% EtOH**. Remove supernatant. Repeat one time.
38. Quick spin tubes with caps facing in. Place on magnet and remove residual EtOH.
39. Take tubes off magnet. Air-dry, caps open, 2-3 min at RT. Beads should be damp matte brown.
40. Add 17 µL/reaction **0.1X TE Buffer**. Pipette/vortex to resuspend beads and quick spin.
41. Incubate 2 min at RT. Quick spin tubes, place on magnet for 2 min.
42. Transfer 15 µL libraries to new **8-strip Tubes.** Proceed to sequencing or store at -20ºC.