Catalog No. 14-1121-24rxn

CUTANA™ ATAC-seq Kit Version 1

QUICK-START CARD

SCAN FOR MANUAL
Read latest manual before first experiment!

Primers not included. For best compatibility, use CUTANA™ Nextera-Compatible CDI Primers (14-1190-24rxn).

SAMPLE PREP (~1.5 HR)

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

	COMPONENT	1rxn	8rxn	12rxn	Storage
1X PBS	10X PBS	30 µL	240 µL	360 µL	On ice for same day use, remainder at RT
	Molecular Grade H_2O	270 µL	2.16 mL	3.24 mL	
Wash Buffer 1	10X Wash Buffer	240 µL	1.92 mL	2.88 mL	On ice for same day use
	Molecular Grade H_2O	2.16 mL	17.3 mL	25.9 mL	
ATAC-seq Nuclei Extraction Buffer	Lysis Buffer	60 µL	480 µL	720 µL	On ice for same day use
	5% Digitonin	0.12 µL	0.96 µL	1.44 µL	
Wash Buffer 2	Wash Buffer 1	1.2 mL	9.6 mL	14.4 mL	On ice for same day use
	10% Tween-20	12 µL	96 µL	144 µL	
Tagmentation Master Mix	2X Tagmentation Buffer	30 µL	240 µL	360 µL	On ice for same day use
	1X PBS	14.3 µL	114.4 µL	171.6 µL	
	5% Digitonin*	0.12 µL	0.96 µL	1.44 µL	
	10% Tween-20	0.6 µL	4.8 µL	7.2 µL	

**For fewer than 8 reactions, dilute Digitonin stock 10-fold in molecular grade water before use.*

2. Dispense 37.5 µL **Tagmentation Master Mix** per reaction to **8-strip Tubes**. Place on ice.
3. For counting, transfer 10 µL starting cells to a new tube, add 10 µL 0.4% Trypan Blue, and mix. Transfer 10 µL to a cell counting slide. Determine cell counts, viability, and cell integrity.
4. Harvest at least 200,000 cells per reaction by centrifugation at 500 x g for 5 min at 4˚C. This ensures sufficient cells are harvested to recover up to 50,000 nuclei per reaction.
5. Carefully remove media without disturbing cell pellet. Resuspend in 100 µL per reaction of **cold 1X PBS**, gently pipetting 5–10 times. Keep cells and nuclei on ice from this point forward.
6. Perform another cell count with Trypan Blue as in Step 3. Transfer 200,000 to 500,000 cold PBS-resuspended cells into 1.5 mL tubes. Keep cells on ice.
7. Spin 500 x g for 5 min at 4˚C. Pipette to remove supernatant without disturbing the pellet.
8. Gently resuspend cells in 1 mL cold **Wash Buffer 1** per 1.5 mL tube.
9. Spin 500 x g for 5 min at 4˚C. Carefully and slowly remove entire supernatant without disturbing the pellet. Keep cell pellet on ice.

NOTE: Nuclei extraction is time sensitive. Perform Steps 10-11 in batches of 4 tubes or fewer to avoid over-lysis.

10. Resuspend cell pellet in 50 µL cold **ATAC-seq Nuclei Extraction Buffer** per tube (≤500,000 cells) by gentle pipetting. Place on ice and incubate for 3 minutes.
11. When 3-minute lysis is complete, quench by adding 1 mL of cold **Wash Buffer 2** per tube. Cap tube and invert a few times to mix before returning to ice.
12. When all samples are lysed and quenched, pellet nuclei by spinning 500 x g for 5 min at 4˚C. Carefully and quickly remove supernatant and place pellet on ice.

13. Resuspend nuclei in 12 µL cold **1X PBS** per reaction. Gently rinse entire side wall of the tube while resuspending. Keep nuclei on ice.
14. For counting, transfer 2 µL nuclei to a fresh tube and add 8 µL **1X PBS**. Perform nuclei count with the 5-fold diluted sample as in Step 3.
15. Use cold **1X PBS** to bring the nuclei concentration down to 5 x 10^6 / mL. This will result in a resuspension that contains 50,000 nuclei per 10 µL for each reaction. If average nuclei per sample type is <5 x 10^6 / mL, calculate actual number of nuclei per 10 µL (see manual).

TAGMENTATION AND CLEANUP (~1.5 HR)

16. To the 8-strip reaction tubes containing **Tagmentation Master Mix** (Step 2), add 2.5 µL **Tn5 Transposome** per reaction. While on ice, gently mix by pipetting.
17. While on ice, add 10 µL nuclei from Step 15. Gently mix by pipetting and quick spin.
18. Incubate tubes at 37˚C for 30 minutes at 1,000 rpm in a thermomixer that is compatible with 8-strip tubes, or in a thermocycler with brief vortexing at 10-min intervals (see manual).
19. Quick spin tubes and add 5 µL **Stop Solution** per reaction. Mix by gentle vortexing for 5 sec and quick spin. Incubate 10 minutes in thermocycler set at 68˚C with heated lid at 105˚C.
20. Quick spin tubes and allow to cool to RT. Prepare 500 µL/reaction 85% EtOH.
21. Vortex **DNA Purification Beads** to fully resuspend. Slowly add 99 µL (1.8X ratio) per reaction.
22. Mix well by vortexing for 5 sec. Quick spin tubes and incubate 5 min at RT.
23. Place tubes on a magnet for 2–5 min. Pipette to remove supernatant without disturbing beads.
24. On magnet, add 180 µL/reaction 85% EtOH. Pipette to remove supernatant. Repeat one time.
25. Quick spin tubes with caps facing in. Return to magnet and pipette to remove residual EtOH.
26. Take tubes off magnet. Air-dry, caps open, 2–5 min at RT. Beads should be damp matte brown.
27. Add 20 µL/reaction **0.1X TE Buffer**. Pipette and/or vortex to resuspend beads and quick spin.
28. Incubate for 2 min at RT. Quick spin tubes and place on magnet for 2 min at RT.
29. Transfer 17.5 µL of eluate to new 8-strip tubes. Proceed to PCR or store at -20˚C.

PCR AMPLIFICATION AND CLEANUP (~1.5 HR)

30. Assign a unique pair of **i5 & i7 Primers** per reaction using **CUTANA™ Nextera-Compatible CDI Primer Set 1** (EpiCypher 14-1190-24rxn, sold separately).
31. To each reaction, add: 6.25 µL **i5 Primer**, 6.25 µL **i7 Primer**, and 30 µL **Non-Hot Start 2X PCR Master Mix**. Gently vortex to mix and quick spin.
32. Perform PCR per table parameters (lid at 105˚C). Prepare 500 µL/reaction 85% EtOH. Remove tubes from thermocycler, quick spin, and allow to return to RT.
33. Vortex **CUTANA DNA Purification Beads** to fully resuspend. Slowly add 108 µL (1.8X ratio) per reaction.
34. Mix well by vortexing for 5 sec. Quick spin tubes and incubate 5 min at RT.
35. Place tubes on a magnet for 2–5 min. Pipette to remove supernatant without disturbing beads.

STEP #	TEMP	TIME	CYCLES
1	72˚C	5 min	1
2	98˚C	30 sec	1
3	98˚C	10 sec	10 (10-15)
4	63˚C	30 sec	
5	72˚C	1 min	
6	4-12˚C	∞	1

36. On magnet, add 180 µL/reaction 85% EtOH. Pipette to remove supernatant. Repeat one time.
37. Quick spin tubes with caps facing in. Return to magnet and pipette to remove residual EtOH.
38. Take tubes off magnet. Air-dry, caps open, 2–5 min at RT. Beads should be damp matte brown.
39. Add 20 µL/reaction **0.1X TE Buffer**. Pipette and/or vortex to resuspend beads and quick spin.
40. Incubate for 2 min at RT. Quick spin tubes and place on magnet for 2 min at RT.
41. Transfer 17.5 µL of eluate per reaction to new 8-strip tubes. Proceed to sequencing or store at -20˚C.