Catalog No. 14-2001-8rxn & 14-2001-24rxn

CUTANA™ Fiber-seq Kit Version 1

QUICK-START CARD

SCAN FOR MANUAL
Read latest manual before first experiment!

SAMPLE PREP (~30 MIN)

1. Prepare buffers as outlined below. Recipes contain 20% excess - no overage is needed. Note that protease inhibitor is not used in the Fiber-seq protocol.

BUFFER	COMPONENTS	1rxn	4rxn	8rxn	STORAGE
Nuclei Extraction Buffer	Pre-Nuclei Extraction Buffer	240 µL	960 µL	1.92 mL	Ice for use
	1 M Spermidine	0.12 µL	0.48 µL	0.96 µL	
1X Reaction Buffer	Pre-1X Reaction Buffer	210 µL	840 µL	1.68 mL	Ice for use
	1 M Spermidine	0.11 µL	0.44 µL	0.88 µL	

2. Collect starting cells by spinning at 600 x g for 3 min at RT. Remove supernatant, flick tube to loosen cell pellet and resuspend pellet in 1-2 mL 1X PBS.
3. Transfer 10 µL cells to a new tube, add 10 µL 0.4% Trypan Blue, and mix. Transfer to a cell counting slide. Determine cell counts, viability (>80%), and cell integrity.
4. Collect 2,000,000 cells/reaction (plus 10% excess if possible). Spin at 600 x g for 3 min at RT. Remove supernatant.

NOTE: 2,000,000 cells/reaction input is recommended for human cells. See Appendix section in the manual for recommendations on cell inputs for other organisms.

5. Resuspend cells in 0.5 mL 1X PBS and transfer to a 1.5 mL tube to wash once.
6. Spin cells at 600 x g for 3 min at RT. Resuspend in 200 µL/reaction cold **Nuclei Extraction Buffer**.
7. Incubate for 10 min on ice.
8. Spin at 600 x g for 3 min at 4˚C.
9. Resuspend nuclei in 75 µL/reaction cold **1X Reaction Buffer**.
10. Take 10 µL nuclei and perform Trypan Blue staining as in Step 3. Obtain nuclei counts and confirm nuclei integrity. Nuclei should be >95% Trypan Blue positive, unclumped, and show minimal lysis.

NOTE: If severe sample clumping is observed, consider counting nuclei in 1X PBS instead for better accuracy: spin extracted nuclei after Step 7 at 600 x g for 3 min at RT, remove supernatant, and thoroughly resuspend nuclei in ~1 mL/reaction cold 1X PBS (supplemented with 0.5 mM Spermidine) before taking 10 µL for Trypan Blue staining and counting. After counting, spin nuclei at 600 x g for 3 min at 4˚C and resuspend with 75 µL/reaction cold **1X Reaction Buffer**.

Take caution as an extra wash step may cause additional loss of nuclei. Having sufficient nuclei (1,000,000 per reaction) is critical to the success of a Fiber-seq reaction.

FIBER-SEQ REACTION (~20 MIN)

11. For each reaction, pipette mix and transfer 1,000,000 nuclei to a PCR tube and bring the volume up to 56.5 µL with **1X Reaction Buffer**. Equilibrate to RT.
12. Add 1.5 µL 32 mM **SAM** to each reaction, pipette gently to mix, followed by addition of 2 µL **Hia5 for Fiber-seq** to initiate each reaction. The final reaction volume per tube should now be 60 µL. Pipette gently to mix.
13. Incubate reaction for 10 min at 25˚C using a thermocycler.

NOTE: The standard Fiber-seq labeling reaction is optimized to achieve ~6% 6mA labeling in 1,000,000 human nuclei. See manual for details on success metrics and scaling guidance.

14. After incubation, stop the reaction by adding 6 µL 10% SDS to each reaction. Vortex to mix.
15. Add 34 µL **1X Reaction Buffer** to bring the volume to 100 µL. Proceed to genomic DNA extraction.

GENOMIC DNA EXTRACTION (~1 HR)

BEFORE FIRST USE PER KIT: Add ethanol (≥95%, not supplied in kit) directly to the **gDNA Wash Buffer** bottle.

For Kit 14-2001-8rxn, add 7 mL ethanol.
For Kit 14-2001-24rxn, add 21 mL ethanol.

16. Add 1 µL **Proteinase K** and 3 µL **RNase A** to each reaction. Vortex to mix.
17. Add 100 µL **gDNA Cell Lysis Buffer** to each reaction. Vortex immediately and thoroughly to mix. The solution will become viscous at this point.
18. Incubate for 30 min at 56°C in a thermomixer with full speed agitation (~1,400 rpm). If a thermomixer is not available, use a heating block and vortex occasionally.
19. Add 400 µL **gDNA Binding Buffer** to each reaction. Pulse-vortex for 5-10 seconds to mix thoroughly.
20. For each reaction, transfer the entire mix (~600 µL) to a **gDNA Spin Column** pre-inserted into a **gDNA Collection Tube**, without touching the upper column area. Proceed immediately to next step.
21. Close cap and centrifuge for 3 min at 1,000 x g to bind gDNA, then for 1 min at max speed (>12,000 x g) without taking tubes out of the centrifuge. Discard the flow through and the collection tube.
22. Transfer each column to a new **gDNA Collection Tube** and add 500 µL **gDNA Wash Buffer.** Close the cap and invert a few times so that the wash buffer touches the cap. Centrifuge immediately for 1 min at max speed (>12,000 x g), then discard the flow through.
23. Reinsert the column into the collection tube. Repeat the wash with 500 µL **gDNA Wash Buffer** per reaction.
24. Place each column in a DNase-free 1.5 mL tube and add 100 µL **gDNA Elution Buffer** preheated at 60˚C. Close cap and incubate for 1 min at RT.
25. Centrifuge at max speed (>12,000 x g) for 1 min to elute the gDNA.
26. Quantify DNA with the Qubit fluorometer and assess gDNA quality on the Agilent TapeStation® using Genomic DNA ScreenTape and Reagents. See manual for example TapeStation traces.
27. Safe pause point. Store DNA at -20˚C, or proceed to LRS library prep and sequencing based on individual platform instructions.