CUT&RUN

Quick Guide

Catalog No. 53180
vA1
Copyright 2024 Active Motif, Inc.

Scan or click for full manual

Prepare Buffers

Prepare DNA Purification Binding Buffer: add 9 mL 100% isopropanol to the DNA Purification Binding Buffer bottle to a final concentration of 60% isopropanol.

Prepare DNA Purification Wash Buffer: add 40 mL of 100% ethanol to the DNA Purification Wash Buffer bottle, the final concentration of ethanol is 80%.

Prepare Stop Solution: Add 11 µL Glycogen to the vial of Stop Solution. Add 22 µL of RNase A to Stop Solution. You will need 40 µL Stop Solution per reaction. Solution will be stable for 6 months at 4°C.

Before beginning, prepare Complete Wash Buffer, Cell Permeabilization Buffer and Antibody Buffer. Some of each buffer will be the base component of the next buffer that follows in sequence in the table below, and the remaining portions of each buffer will be stored as indicated in this table and used as indicated in the protocol.

Complete Nuclei Isolation Buffer will be needed if following the optional but recommended Nuclei Preparation steps below: Add 1.4 µL 1 M Spermidine to the Nuclei Isolation Buffer bottle. When ready to use in the assay, add 10 µL 100X Protease Inhibitor Cocktail per 1 mL Nuclei Isolation Buffer. 100 µL Complete Nuclei Isolation Buffer are needed per sample.

Buffer Name	Components	1 rxns	8 rxns	24 rxns	Storage
Complete Dig-Wash Buffer	Dig-Wash Buffer	1.8 mL	14.4 mL	43.2 mL	4°C, for use on Day 1
	Protease Inhibitor Cocktail (100X)	18 µL	144 µL	432 µL	
	1 M Spermidine	0.9 µL	7.2 µL	21.6 µL	
Cell Permeabilization Buffer	Complete Dig-Wash Buffer	1.4 mL	11.2 mL	33.6 mL	4°C, for use on Day 2
	5% Digitionin	2.8 µL	22.4 µL	67.2 µL	
Antibody Buffer	Cell Permeabilization Buffer	100 µL	800 µL	2.4 mL	Ice, for use on Day 1
	0.5 M EDTA	0.4 µL	3.2 µL	9.6 µL	

For nuclei isolation, (Step 13, optional but recommended) prepare Complete Nuclei Isolation Buffer: You will need 100 µL per sample. Add 1.4 µL 1 M Spermidine to the Nuclei Isolation Buffer bottle. When ready to use in the assay you will need to Add 10 µL 100X Protease Inhibitior Cocktail (PIC) per 1 mL Nuclei Isolation Buffer.

Day 1 Prepare Concanavalin A Beads

1. Resuspend Concanavalin A Beads by vortexing on low setting.
2. Add 10 µL to each empty sample tube.
3. Place tubes on magnet to clear, remove supernatant and discard.
4. Add 100 µL 1X Binding Buffer per sample.
5. Pipette to resuspend, place tubes on magnet, remove supernatant and discard.
6. Repeat steps 4 and 5 once more for a total of two washes.
7. Add 10 µL of Binding Buffer to each sample and store on ice.

Prepare Cells (5,000 to 500,000 freshly grown, or thaw cryopreserved cells)

8. Centrifuge cells at 600 x *g* for 3 minutes at room temperature. Remove and discard supernatant.

Prepare Nuclei (optional but recommended)

9. Gently resuspend cell pellets in 100 µL Complete Nuclei Isolation Buffer per up to 500,000 cells. This volume does not need to scale down for less than 500,000 cells.
10. Gently pipette or flick tube to ensure pellet is fully resuspended.
11. Place nuclei samples on ice for 10 minutes.
12. Centrifuge nuclei at 600 x *g* for 3 minutes at room temperature. Remove and discard supernatant.

Wash Cells/Nuclei

13. Gently resuspend cell or nuclei pellets in 100 µL Complete Dig-Wash Buffer per sample.
14. Centrifuge cells or nuclei at 600 x *g* for 3 minutes at room temperature.Remove and discard supernatant.
15. Repeat steps 13 – 14 once more for a total of 2 washes.
16. Add 100 µL of Complete Dig-Wash Buffer per sample and gently resuspend.
17. Transfer 100 µL of washed cells/nuclei to prepared Concanavalin A Beads.
18. Resuspend beads and cells/nuclei by gently pipetting up and down.
19. Allow to sit at room temperature for 10 minutes.
20. Place tubes on magnet or magnetic rack. Remove and discard supernatant.
21. Add 50 µL of Antibody Buffer to each sample.

Antibody Binding

22. Add 1 µg or 1 µL of appropriate antibody to each sample. Resuspend by gently pipetting up and down. Include CUT&RUN Spike-In Nuclei and Antibody if desired.
23. Place samples on Nutator or orbital shaker overnight at 4°C at speed of 20-30 RPM.

For additional help, watch:
Epi Insider: Working with Con A Beads in CUT&RUN and CUT&Tag Assays

Day 2 Washes

1. Quick spin tubes and place tubes on magnet or magnetic rack at room temperature.
2. Remove and discard supernatant.
3. Add 200 µL Cell Permeabilization Buffer to each sample without disturbing pellet.
4. Remove and discard supernatant.
5. Repeat steps 3 - 4 once more for a total of 2 washes.

ChIC/CUT&RUN pAG-MNase Binding

6. Add 50 µL Cell Permeabilization Buffer to each sample.
7. Add 2.5 µL of ChIC/CUT&RUN pAG-MNase to each sample.
8. Remove tubes from magnet. Resuspend beads and mix by gently pipetting up and down.
9. Allow to sit at room temperature for 10 minutes.
10. Place tubes on magnet or magnetic rack. Remove and discard supernatant.
11. Add 200 µL Cell Permeabilization Buffer to each sample without disturbing pellet.
12. Remove and discard supernatant.
13. Repeat steps 12 - 13 for a total of 2 washes.
14. Add 50 µL Cell Permeabiliaztion Buffer to each sample.
15. Place tubes on ice and pause for 2 minutes to allow samples to cool before proceeding to the next steps.

Targeted Chromatin Digestion

16. Add 1 µL of 0.1 M $CaCl_2$ to each sample.
17. Mix by gently pipetting up and down to resuspend beads and place back on ice.
18. Place on Nutator or orbital shaker at 4°C for 2 hours at speed of 20-30 RPM.
19. Remove from Nutator or orbital shaker and place samples on ice.
20. Add 40 µL of Stop Solution to each sample. Mix samples by gently pipetting up and down.
21. Incubate samples at 37°C for 10 minutes in a thermal cycler with lid set to 65°C.
22. Place tubes on magnet or magnetic rack for at least 30 seconds.

ChIC/CUT&RUN DNA Isolation and Purification

23. Carefully transfer samples to fresh 1.5 mL microcentrifuge tubes, avoiding any beads.
24. Be sure that fresh isopropanol has been added to the bottle of DNA Purification Binding Buffer to a final percentage of 60% to DNA Purification Binding Buffer before this step. To each sample tube, add 450 µL of DNA Purification Binding Buffer.
25. Vortex briefly and let stand 1 minute at room temperature.
26. Transfer sample and buffer to DNA Purification Columns SF.
27. Centrifuge at 16,000 x *g* for 1 minute. Pour off flow through.
28. Add 200 µL DNA Purification Wash Buffer to each column.
29. Centrifuge columns at maximum speed (16,000 x *g*) for 1 minute.
30. Repeat steps 31 - 32 for a total of 2 washes. Pour off flow through.
31. Centrifuge at maximum speed (16,000 x *g*) for 1 minute to ensure all traces of DNA Purification Wash Buffer are removed.
32. Transfer column to new collection tube.
33. To each column, add 55 µL DNA Purification Elution Buffer. Let stand at room temperature for 1 minute.
34. Centrifuge at maximum speed (16,000 x *g*) for 1 minute.
35. Transfer eluted ChIC/CUT&RUN DNA to new tubes. Store at -80°C if not being used immediately for NGS Library Preparation. These samples are now ready for NGS Library Preparation.