

**DAY 1****Section I: CUT&RUN Buffer Preparation (~30 min)**

1. Prepare buffers (see Table below). Optimize Digitonin for each cell type (see manual **Appendix 1.1**).

BUFFER NAME	COMPONENTS	1 RXN	8 RXN	16 RXN	STORAGE
Wash Buffer	Pre-Wash Buffer	1.8 mL	14.4 mL	28.8 mL	RT for use on Day 1
	25X Protease Inhibitor	72 µL	576 µL	1.15 mL	
	1 M Spermidine	0.9 µL	7.2 µL	14.4 µL	
Cell Permeabilization Buffer	Wash Buffer	1.4 mL	11.2 mL	22.4 mL	4°C for use on Day 2
	5% Digitonin	2.8 µL	22.4 µL	44.8 µL	
Antibody Buffer	Cell Perm. Buffer	100 µL	800 µL	1.6 mL	Ice for use on Day 1
	0.5 M EDTA	0.4 µL	3.2 µL	6.4 µL	

Section II: ConA Bead Activation (~30 min)

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. Remove tube from magnet and immediately 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.

Section III: Binding Cells to Activated Beads (~30 min)

5. Count starting cells and confirm integrity and viability. Harvest 500,000 cells/reaction (plus 10% excess). Spin at 600 x g for 3 min at room temperature (RT). Pipette to remove supernatant.
6. Resuspend cells in 100 µL/reaction RT **Wash Buffer**. Spin 600 x g, 3 min, RT. Remove supernatant. Repeat one time.
7. Resuspend cells in 105 µL/reaction RT **Wash Buffer**. Count and examine integrity of prepared cells.
8. Add 100 µL cells to 10 µL **activated ConA beads** in 8-strip tubes. Gently vortex to resuspend and quick spin in a mini-centrifuge to collect liquid. Incubate 10 min at RT to adsorb cells to beads.
9. If using a multi-channel pipettor, place a reagent reservoir on ice. Fill with cold **Antibody Buffer**. **Note:** Remove and replace buffers one tube strip at a time to avoid ConA bead dry-out and sample loss.
10. Place tubes on a magnet and allow slurry to clear. Pipette to remove supernatant; use 10 µL for Trypan Blue staining to confirm that cells are not in supernatant (**Appendix 1.2**).
11. Remove tubes from magnet. Immediately add 50 µL/reaction cold **Antibody Buffer** and pipette to resuspend. Take a 10 µL aliquot to confirm ConA bead binding (**Appendix 1.2**).

Section IV: Antibody Binding (~30 min + overnight)

12. Quick spin the **K-MetStat Panel** stock and mix by pipetting (do **NOT** vortex stock). To reactions designated for H3K4me3 and IgG Control Antibodies, add 2 µL **K-MetStat Panel** and vortex to mix. **Note:** If using <500,000 cells, decrease K-MetStat Panel per instructions on p. 16 of the manual.
13. Add 0.5 µg antibody to each sample. For designated control reactions, add 1 µL **H3K27me3 Positive Control Antibody** and 1 µL **IgG Negative Control Antibody**. Vortex to mix and quick spin.
14. Incubate overnight on a nutator at 4°C, gently rocking tubes with caps slightly elevated. Do **NOT** rotate tubes end-over-end, as this will result in sample loss.



DAY 2

Section V: Binding of pAG-MNase (~40 min)

- Place a reagent reservoir on ice. Fill with cold **Cell Perm. Buffer**.
- Remove tubes from 4°C, quick spin to collect liquid. **Note:** Beads may settle overnight; this is normal.
- Place tubes on a magnet and allow slurry to clear. Pipette to remove supernatant.
- Keep tubes on magnet. Add 200 µL/reaction cold **Cell Perm. Buffer**. Remove supernatant. Repeat one time.
- Remove tubes from magnet. Add 50 µL/reaction cold **Cell Perm. Buffer** and gently vortex to mix. **Note:** Beads may become clumpy at this stage of the protocol. Disperse by gentle pipetting.
- Add 2.5 µL/reaction **pAG-MNase**. Gently vortex or pipette to mix beads and distribute enzyme.
- Quick spin tubes and incubate 10 min at RT.
- Quick spin tubes, place on magnet, and allow slurry to clear. Remove supernatant.
- Keep tubes on magnet. Add 200 µL/reaction cold **Cell Perm. Buffer**. Remove supernatant. Repeat one time.
- Remove tubes from magnet. Add 50 µL/reaction cold **Cell Perm. Buffer**. Gently vortex to mix and disperse clumps by pipetting. Quick spin tubes to collect liquid and place on ice.

Section VI: Targeted Chromatin Digestion and Release (~3 hrs)

- Add 1 µL/reaction **100 mM Calcium Chloride**, and gently vortex or pipette to evenly resuspend.
- Quick spin and incubate tubes (caps slightly elevated) on a nutator for 2 hours at 4°C.
- Prepare **Stop Master Mix**: per reaction, combine 1 µL **E. coli Spike-in DNA** and 33 µL **Stop Buffer**. Gently vortex to mix. **Note:** If using <500,000 cells, see **Appendix 2** for instructions.
- At end of incubation, add 34 µL/reaction **Stop Master Mix**. Gently vortex to mix and quick spin.
- Place reactions in a thermocycler set to 37°C. Incubate for 10 min.
- Quick spin tubes, place on magnet, and allow slurry to clear. Transfer supernatants containing CUT&RUN-enriched chromatin to new **8-strip tubes**. Discard tubes with ConA beads.

Section VII: DNA Purification (~30 min)

- Prepare 500 µL 85% Ethanol (EtOH) per reaction by combining 425 µL 100% EtOH and 75 µL molecular biology grade water. Prepare **fresh**, mix well, and store at RT.
- Vortex **SPRIselect** reagent (manufactured by Beckman Coulter, Inc.*) to thoroughly resuspend beads. Slowly add 119 µL/reaction.
- Mix well by pipetting and/or vortexing to resuspend. Quick spin to collect liquid. Incubate 5 min at RT.
- Place tubes on magnet for 2-5 min. Pipette to remove supernatant without disturbing beads.
- Keep tubes on magnet. Add 180 µL/reaction **85% EtOH**. Remove supernatant. Repeat one time.
- Quick spin with caps facing in to avoid dislodging beads. Return to magnet, remove residual EtOH.
- Remove tubes from magnet and air-dry, caps open, for 2-3 min at RT. Beads should appear damp matte brown (**Figure 1**). If beads are crackly/light brown, they are too dry.
- Add 17 µL/reaction **0.1X TE Buffer** to elute DNA. Pipette or vortex to resuspend. Incubate 2 min, RT.
- Quick spin tubes and place on magnet for 2 min. Transfer 15 µL CUT&RUN DNA to new **8-strip tubes**.
- Use 1 µL to quantify DNA with the Qubit fluorometer. Proceed to library prep using the CUTANA™ CUT&RUN Library Prep Kit (EpiCypher 14-1001 & 14-1002) or store DNA at -20°C.

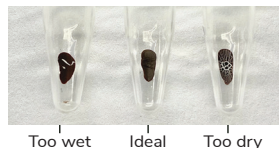


FIGURE 1
Elute DNA when "ideal."

Do **NOT** examine CUT&RUN DNA on the TapeStation/Bioanalyzer, as DNA yields are too low. Wait until **after library prep** to examine fragment distribution.