# Fixed ATAC-Seq Sample Preparation Protocol

vA1



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## Reagents & Buffers

**Formaldehyde:**

For best results, use fresh, methanol-free formaldehyde. We recommend using 16% formaldehyde such as Pierce™ 16% Formaldehyde (w/v), Methanol-free, Catalog No. 28906, available from Thermo Fisher Scientific.

**Cell Fixation Buffer:**

| Compound | Initial Concentration | Final Concentration |
|---|---|---|
| Formaldehyde Solution (formaldehyde must be added right before using if preparing your own) | 16% | 11% |
| NaCl | 5 M | 100 mM |
| EDTA, pH 8.0 | 250 mM | 1 mM |
| HEPES, pH 7.5 | 1 M | 50 mM |
| Water | Quantity Sufficient | Quantity Sufficient |

**Note:** Add formaldehyde to the Cell Fixation Buffer **immediately before use.**

## Protocol

We recommend performing fixation in suspension to allow for accurate cell counting. This protocol assumes cells are already in suspension and does not include a detachment step.

1. Bring the cell suspension concentration to 1 - 2 x $10^6$ cells/mL with complete cell culture medium in a 15 mL tube (<10 mL of suspension) or 50 mL tube (10 - 30 mL of cells). If <1 x $10^6$ cells, use 0.5 mL of the medium in a 1.5 mL tube.
2. Gently vortex the cell suspension, add 1:10 (vol:vol) 10X Cell Fixation Buffer drop by drop with a pipette and rotate at a low speed for 10 minutes at room temperature in an orbital shaker.
3. Quick spin the tubes and stop the reaction by gently vortexing, and adding 2.5 M glycine in the ratio of 1:20 (vol:vol). Invert the tubes 2-3 times and incubate on ice for 5 minutes.
4. Perform the remaining steps at 4°C or on ice. Spin the tubes at 800 x *g* for 5 minutes at 4°C in a swinging bucket centrifuge and discard the supernatant.
5. Resuspend the pellet gently with 5 mL of ice-cold 1X PBS and incubate for 2 minutes on ice.
6. Repeat steps 4 and 5 with a volume of 1 mL of ice-cold 1X PBS that will bring the cell density to 1 x $10^6$ cells/mL. Aliquot 350,000 cells into precooled 1.5 mL tubes labeled for long-term storage.

**Note:** We recommend aliquoting cells into 350,000 cell pellets. Cell pellets are then snap frozen and can be stored at -80°C until you are ready to ship your samples.

7. Spin the tubes at 1,200 x *g* at 4°C and remove as much of the supernatant as possible without disturbing the cell pellet. Snap freeze the tubes in liquid nitrogen, and store at -80°C.

**Best Practices for Shipping Samples to Active Motif**

- Include ample dry ice to keep samples frozen during shipment.
- Avoid shipping over weekends or for Saturday delivery.
- Ship samples Monday through Wednesday to ensure timely delivery.