

scATAC-Seq Sample Preparation

We recommend preparing between **100,000 – 2,000,000 cryopreserved cells for scATAC-Seq**. Higher amounts of cells increase the chance of success. The cell preparation protocols below have been optimized for >2 million cells. If working with <500,000 cells, adjust wash step volumes and tube sizes (i.e. from 15ml conical to 1.5 mL Eppendorf tube) to minimize cell loss.

If using primary cell cultures, treating cells with compounds that may induce cell death, or cells that have been genetically modified in a way that may induce cell death, follow instruction for DNase treatment prior to preparing your cells for shipment.

For the scATAC-Seq service, a sample validation is required prior to full scale project. For sample validation, submit sample in duplicate and prepared identically to the experimental sample(s). This requirement can be forgone only under special circumstances with approval.

If the project consists of samples with different experimental conditions (e.g. time course, different drug treatments), and you need to select one condition for sample validation and need to perform DNase treatment, we recommend selecting the condition that is predicted to be the most damaging to the sample.

I. DNase Treatment

If using healthy cultured cell lines, or primary cells that are not amenable to being in culture conditions (e.g. primary cells isolated from tissues) skip DNase treatment step and start protocol at step 1 of Cryopreservation section.

Reagents needed for DNase treatment:

- DNase (Worthington, LS002006)
Other manufacturers can be used if it can be added in the appropriate concentration to the cell culture.
- HBSS (Gibco, 14025-092)

Solutions to make for DNase treatment:

- 100x DNase solution: Resuspend DNase in HBSS at 20,000 units/mL. Freeze at -20°C or use fresh.
- 100x DNase buffer: 250mM MgCl₂ and 50mM CaCl₂ in dH₂O

For adherent cells:

1. Wash cells (remove existing growth media, add fresh pre-warmed media*, aspirate, add media again). *Use media that supports healthy growth specific to cell type
2. Add 1:100 volume of 100x DNase buffer and 1:100 volume of 100x DNase solution to media.

3. Incubate at 37°C for 30 minutes in tissue culture incubator.
4. Aspirate media, wash with PBS, and detach cells using dissociation method specific to your cell type/line. Following dissociation, transfer cells to a 15mL or 50mL conical tube. Centrifuge at 500 x g for 5 minutes at 4°C to pellet the cells, aspirate media, resuspend cell pellet in 5mL[§] of ice-cold PBS.
§ If working in 1.5 mL Eppendorf tubes, adjust wash volume to 500 µL of ice-cold PBS.
5. Quantify cells using hemocytometer or equivalent method to determine volume needed to achieve proper concentration for cryopreservation. Document viability if possible. While quantifying, keep the cells on ice.
6. Proceed to Cryopreservation section starting at step 3.

For suspension cells:

1. Transfer cells to a 15mL or 50mL conical tube.
2. Centrifuge at 500 x g for 5 minutes to pellet the cells. Aspirate existing growth media and resuspend in 10 ml of pre-warmed fresh growth media*.
**Use media that supports healthy growth specific to cell type*
3. Add 1:100 volume of 100x DNase buffer and 1:100 volume of 100x DNase solution to media, mix by inverting the tube several times.
4. Loosen the lid on the conical tube, return to incubator and incubate at 37°C for 30 minutes.
5. Centrifuge at 500 x g for 5 minutes at 4°C to pellet the cells, aspirate media, resuspend cell pellet in 5mL[§] of ice-cold PBS.
§ If working in 1.5 mL Eppendorf tubes, adjust wash volume to 500 µL of ice-cold PBS.
6. Quantify cells using hemocytometer or equivalent method to determine volume needed to achieve the proper concentration for cryopreservation. Document viability if possible. While quantifying, keep the cells on ice.
7. Cryopreserve cells according to the protocol below starting at step 3.

II. Cryopreservation: (if skipping the DNase treatment, start here)

1. Incubate Mr. Frosty or equivalent device at 4°C for a minimum of 1-hour prior to use.
2. For healthy adherent cells lines, enzymatically detach them using trypsin or another enzyme as needed for your specific cell type. For healthy suspension cells, transfer cells in growth media to a conical tube for pelleting.

3. Centrifuge at 500 x g at 4°C to pellet the cells and remove supernatant.
4. Resuspend cells in the appropriate volume of ice-cold cryopreservation solution – 50% FBS/40% growth media/10% DMSO – to achieve a concentration of 4 million cells/mL. If there are less than 2 million cells total, use 500 µL. Transfer 500 µL to a 1.5 mL Eppendorf tube on ice. **Do not use screw cap or cryotubes that cannot fit into a microcentrifuge.**
5. Freeze the cells by transferring the tubes to a pre-chilled Mr. Frosty container or equivalent device, like the one depicted below and place at -80° C.



6. If necessary, an alternate approach is to place the tubes upright in a styrofoam container. Close the styrofoam container with the styrofoam top and then place at -80° C.
7. Ship cells on dry ice. Fill out Active Motif's [Sample Submission Form](#), include a hard copy with your samples and ship according to the instructions on the Sample Submission Form.

For Tissues:

If you are submitting tissues for scATAC-Seq, freeze the tissue according to one of the protocols below. Tissue requirements for scATAC-seq are 20 to 50 mg.

Liquid Nitrogen (preferred method)

1. Excise the tissue from the animal and place in a microfuge tube.
2. Submerge in liquid nitrogen for 2 minutes.
3. Store at -80 °C.

Dry Ice

1. Excise the tissue from the animal and place in a microfuge tube.
2. Place tube into a dry ice bath with ethanol for 15 minutes.
3. Store at -80 °C.

For Organoids and Very Small Tissues (<10mm³):

Organoids and very small tissues (eg. pancreatic islets, embryonic tissues, tissues only several cell layers thick such as retina or epidermis) should be cryopreserved in 500 µL of cryopreservation solution – 50% FBS/40% growth media/10% DMSO as if they were cells, aiming for > 2 million cells total. Standalone DNase treatment is not recommended. However, if an enzymatic isolation or microdissection step is utilized, it may be possible to incorporate DNase treatment during this step (even at room temperature or on ice). If DNase treatment is incorporated, the organoids/tissues should be washed prior to cryopreservation to minimize DNase carryover.