

ATAC-Seq Sample Preparation

We recommend preparing 100,000 cells for ATAC-Seq. Please do not send more than 100,000 cells. If cells are limited, 50,000 cells is also acceptable.

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. However, if samples undergo FACS or similar sorting, please skip DNase treatment.

Tumor tissues are known to often produce lower quality data and may need additional sample processing to improve data quality. Please consult with your sales representative for more information.

For ATAC-Seq 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.

Projects with multiple experimental conditions (e.g. time course, different drug treatments, etc.) can use a single validation and 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 Section II, Cryopreservation.

Reagents needed for DNase treatment:

- DNase (Worthington, LS002006)
Other product substitutes can be used as long as 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 the proper concentration of 100,000 cells 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 of 100,000 cells 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 500 µL of ice-cold cryopreservation solution – 50% FBS/40% growth media/10% DMSO. Transfer 500 µL to a 1.5 mL Eppendorf or 2 mL cryotube on ice.

- 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 overnight to complete cryopreservation.



- 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 .
- 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.

Tissue preparation protocol

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

Liquid Nitrogen

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

Dry Ice

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

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

Organoids, very small tissues (eg. pancreatic islets, embryonic tissues, tissues only several cell layers thick such as retina or epidermis), and rare or very delicate tissues should be cryopreserved in 500 μL of cryopreservation solution – 50% FBS/40% growth media/10% DMSO as if they were cells, aiming for 100,000 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 with PBS prior to cryopreservation to minimize DNase carryover.