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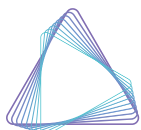
From Azenta Life Sciences

GUIDE

Single-Cell Sequencing Sample Preparation Protocol



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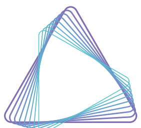

Introduction

Depending on the goals of your single-cell sequencing experiment and the library preparation chemistries to be utilized, cells and tissues may be fixed by paraformaldehyde or cryopreserved in a DMSO-based cryopreservation buffer. This document outlines sample preparation workflows for tissues and cells prior to shipment to Azenta Life Sciences for processing.

As a general guide and if it aligns with your project goals, Azenta recommends fixing cells in paraformaldehyde as it circumvents challenges associated with poor cell viability that may be encountered when working with fragile primary cells. However, as shown in Table 1, paraformaldehyde fixation is not compatible with all library or sample types.

Sample Preservation Workflow	Cryopreservation	Fixation
Supported 10X Genomics Chemistries	• 3' Gene Expression • 5' Gene Expression • V(D)J Immune Profiling • Cell surface protein • scATAC-seq • Multiome ATAC + Gene Expression	• Fixed RNA Profiling • Cell surface protein
Supported Species	• Species agnostic (all polyA+ transcripts)	• Probe-based (human & mouse only)
Minimum Cell Number	• Recommended: 1M cells • Required: 50K cells	• Recommended: ≥ 500K cells • Required: 300K cells

Table 1. Summary of sample preservation compatibility

Once the sample preservation approach has been determined, refer to Figure 1 for the applicable sample preparation section to reference.

For further assistance to design your experiment and decide on the optimal approach, please contact NGS@azenta.com.

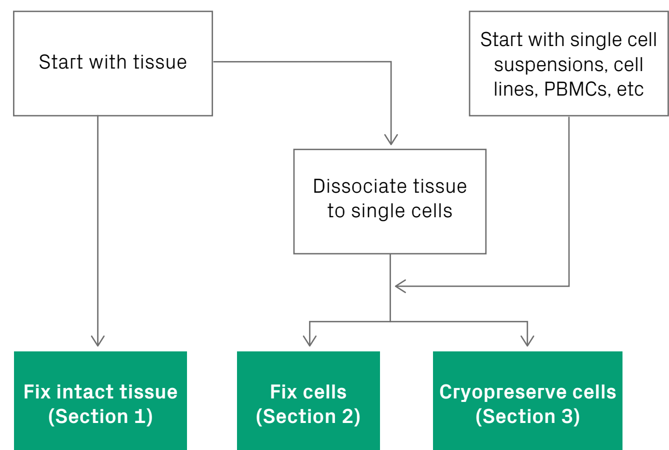

Figure 1. Sample preservation decision tree

1 Fixation of Intact Tissue

USEFUL TIPS BEFORE BEGINNING:

- Note that 25mg of tissue is required
- Testing the fixation workflow in a pilot experiment for different tissue types is recommended
- Be sure to review fixed tissue and cell suspension storage guidelines and confirm that it matches the expected timelines for shipping out
- It is recommended to keep the sample fixation time constant across samples within an experiment

1. Follow demonstrated Tissue Fixation and Dissociation protocol from 10X Genomics®.
2. After addition of 1 ml Tissue Resuspension Buffer, resuspend the tissue pellet, and maintain on ice.
3. Add 0.1 volume of pre-warmed Enhancer (short-term storage at 4°C), and immediately ship to Azenta Life Sciences on cold packs.

2 Fixation of Dissociated Cells and Nuclei

USEFUL TIPS BEFORE BEGINNING:

- Note that ≥300,000 cells or ≥500,000 nuclei are required
- Avoid submitting samples with clumps of cells or nuclei
- Use wide bore tips and resuspend thoroughly with gentle pipetting
- Whenever possible, analyze cells with an automated cell counter and use fluorescent or visible dyes to improve estimation of live and dead cell counts prior to fixation
- It is recommended to keep the sample fixation time constant across samples within an experiment

1. Follow demonstrated Fixation of Cells & Nuclei protocol from 10X Genomics®.
2. After quenching the fixation reaction, immediately proceed to long-term storage at –20°C or –80°C. It is recommended to store the samples at –80°C prior to shipping to Azenta Life Sciences on dry ice.

3 Cryopreservation of Dissociated Cells

USEFUL TIPS BEFORE BEGINNING:

- Many cell types will require optimization of the cryopreservation media for optimal results
- Avoid submitting samples with clumps of cells or tissue
- Use wide bore tips and resuspend thoroughly with gentle pipetting
- Use 2mL cryovials during cryopreservation to minimize cell damage
- Do not overfill cryovials (no more than 1mL in 2mL vial)
- FACS counts can overestimate cell number (for best results, follow step 3 below)
- DMSO can be toxic at room temperature (use a DMSO-free media until ready to begin cell freezing)

NUMBER OF CELLS TO SUPPLY:

- Recommended: 1M cells in 500µl
- Minimum: 50,000 viable cells in 500µl
- For Dead Cell Removal: 500,000-1M cells in 500µL

CELL FREEZING PROTOCOL:

1. Prepare cryopreservation medium using the table below as a guide. Maintain at 4°C throughout use.

Cell Type	Typical Media	% Media	% Serum (FBS)	% DMSO
PBMCs	RPMI	0-50%	40-90%	10%
Stem Cells, Cancer Lines, Suspension Cell Lines	DMEM	0-50%	40-90%	10%
Adherent Cell Lines	None	0%	90%	10%
Fragile/Primary Cells	None	0%	90%	10%

Table 2. Demonstrated cryopreservation media components

Note: The composition of a standard cryopreservation medium which is suitable for many cell types is provided in Table 2. However, cryopreservation medium and cell handling may need to be optimized for some cell types to ensure that the recommended >90% viability can be obtained after thawing.

3 Cryopreservation of Dissociated Cells *cont.*

2. Prepare a single-cell suspension, and store at 4°C during counting:
   a. For plated cells, collect cells and centrifuge to pellet. Resuspend cell pellet in growth medium and gently pipette up and down with a wide bore pipette 10 times to mix. Strain cells to remove clumps.
   b. For low volume cell suspensions, a Flowmi™ Tip Strainer is recommended for minimal loss of sample volume.
   c. For tissues, enzymatic or mechanical homogenization is recommended to yield a single-cell suspension. Strain tissue homogenates or clumping cells before counting to increase accuracy.

3. Count cells:
   a. Obtaining an accurate cell count is vital. If the concentration is too high to achieve an accurate cell count, dilute aliquots and recount.
   b. Whenever possible, analyze cells with an automated cell counter.
   c. Use fluorescent or visible dyes to improve estimation of live and dead cell counts.
   d. Store multiple aliquots of counted cells if available.

4. Centrifuge and carefully remove supernatant without disturbing the cell pellet. Gently re-suspend cells in chilled cryopreservation medium using a wide bore pipette as follows:
   a. If providing 1M cells (recommended), re-suspend cells to achieve a concentration of $2x10^6$ cells/ml and aliquot 500µl per cryovial.
   b. If providing the minimum number of cells (50,000), re-suspend cells to achieve a concentration of $1x10^5$ cells/ml and aliquot 500µl per cryovial.

5. Place cryovials in the Chill CoolCell® FTS30 (or equivalent) and place in a -80°C freezer for at least 4 hours.

6. After ≥4 hours, pack samples on dry ice for transport to Azenta Life Sciences, or store in liquid nitrogen for long-term storage.

7. When submitting samples with unknown quality, we recommend freeze-thaw testing single aliquots from each sample condition. This step is not necessary - but is highly recommended - for cell types that have known freeze-thaw resilience:
   a. Remove sample from -80°C or liquid nitrogen and thaw rapidly in a 37°C water bath with gentle shaking for 1 minute.
   b. When a small ice crystal remains, remove samples from 37°C and resuspend in 5mL of 37°C media. Transfer suspension to a 15mL tube and centrifuge to pellet cells.
   c. Remove supernatant and resuspend cells in 1mL of PBS or appropriate counting buffer.
   d. Count cells and confirm samples meet sample submission requirements after thawing.

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