Sample Fixation for Single Cell RNA Sequencing

Protocol

Legal Notices

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Required Materials

Consumables and Reagents Manufactured by ScaleBio

ScaleBio Sample Fixation Kit (PN 2020001) Consumables and Reagents.

Kit Module	Consumable	Part Number	Quantity	Storage Temperature
Module A (PN 2020002)	Wash Buffer	202100001	12	–20°C
Module B (PN 2020003)	Fixation Reagent	202110001	12	4°C

Consumables and Reagents Manufactured by Other Vendors

Consumable or Reagent	Supplier	Part Number
1X PBS without calcium or magnesium	Various	Various
DEPC (purity ≥96%)[a]	Millipore Sigma	D5758-25ML
DMSO (anhydrous)[a]	Thermo Fisher	D12345
Methanol (purity ≥99.9%)[a]	Fisher Scientific	A412-500
Pipette tips (nuclease-free, filtered, low retention for P1000, P200, P20)[b]	Various	Various
Pipette tips (nuclease-free, filtered, wide bore for P1000)[b]	Various	Various
15-mL conical tubes[b]	VWR	10025-286
5-mL DNA LoBind tubes[b]	Eppendorf	0030108310
1.5-mL DNA LoBind tubes[b]	Eppendorf	0030108418
Cell strainer (optional)[c]	VWR	10032-802
Cell counting dye [before fixation: trypan blue, after fixation: AO/PI, YOYO-1]	Various	Various

a. Other vendors may be used if reagent formulation remains the same.
b. Required for best assay performance.
c. Type and filter size appropriate for sample (ie, cell diameter and cell suspension volume).

Recommended Equipment

Item	Supplier	Part Number
Centrifuge with temperature control (1.5-mL tubes)	Various	Various
Vortex mixer	Various	Various
Pipettes (P1000, P200, P20, P10)	Various	Various
Cell counter	Various	Various
Chemical fume hood	Various	Various

Best Practices

For general laboratory practices:

- Calibrate and service pipettes every 12 months to ensure accurate sample volume transfer at each step.
- Store all reagents at the storage conditions recommended by the supplier.
- Thaw all reagents on ice, unless otherwise specified.
- Unless otherwise specified, vortex reagents.
- Open Fixation Reagent packing in a chemical fume hood.
- Handle Fixation Reagent, DEPC, and methanol in a chemical fume hood.
- Never reuse pipette tips or tubes.
- Use wide-bore tips for pipetting cell suspensions.
- Keep pipette tip boxes, reagent containers, and sample tubes closed when not in use.
- Wear suitable protective clothing, eyewear, and gloves.

For RNase-free sample processing:

- Use low-retention, RNase-free pipette tips and low-binding reaction tubes to prevent adsorption to plastic surfaces.
- Routinely wipe work surfaces with RNase AWAY to remove RNases, and with a 10% bleach cleaning solution to remove DNA amplicon contaminants.
- Wear disposable gloves and change them frequently.

Assay Introduction

The ScaleBio™ Sample Fixation Kit (PN 2020001) is intended for fixing cells prior to use with the ScaleBio RNA Sequencing Kit. Fixation can be performed at different timepoints, as samples can be stored for up to 12 months at −80°C prior to use, reducing batch effects during library preparation.

Cell Preparation

Review the following table to prepare reagents for starting this section.

Source	Material
Other vendors	• **1X PBS (without calcium or magnesium)** • **15-mL conical tubes** • **Pipette tips (nuclease-free, filtered, wide bore for P1000)** • **Cell counting dye** • **Cell counter** • **Cell strainer (optional)**

IMPORTANT: Use wide-bore pipette tips, and gentle pipette-mixing, for preparing cell suspensions to maintain sample quality. Cells must be washed with 1X PBS and resuspended in a maximum volume of 50 µL prior to fixation.

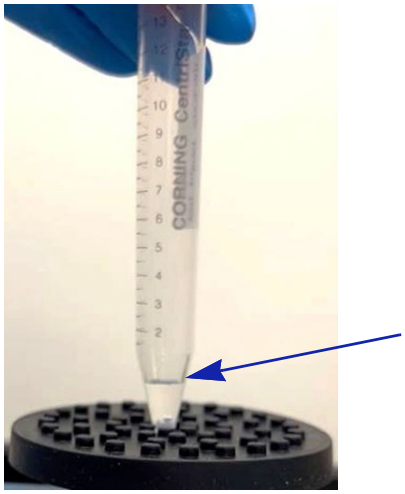
NOTE: If the initial cell numbers range from 100,000 to 1 million cells, contact your Field Application Scientist for suggestions on protocol modifications for lower cell numbers.

Before you Begin

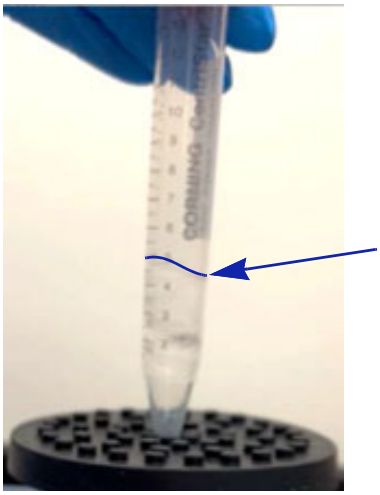
- Place 1X PBS on ice.
- Bring centrifuges (for 15-mL tubes) to 4°C.
- Start with sufficient cells to obtain 1 million to 2.5 million cells after washing and counting, assuming cell loss throughout the process.
- Determine the speed setting for the vortex mixer that will be used during the fixation process, using a 15-mL conical tube containing 500 µL of water. The speed of the vortex mixer should be set such that the height of the 500 µL water reaches the 5 mL mark on a 15-mL conical tube as shown:

Figure 1: Setting the vortex speed

500 µL of water without vortexing



500 µL water while vortexing



Procedure

1. Obtain cells from culture, tissue dissociation, or thaw if frozen, and place **on ice**.
2. Transfer the cell suspension into a 1.5-mL conical tube.
3. Centrifuge the cells at **500 x g** for **5 minutes** at **4°C**.

   NOTE: Nuclei or small cells can be centrifuged for 8–10 minutes to maximize recovery.
4. Carefully remove the supernatant without disturbing the pellet.
5. Add **500 µL** ice cold 1X PBS and resuspend the cells by flicking.
6. Centrifuge the cells at **500 x g** for **5 minutes** at **4°C**.
7. Carefully remove the supernatant without disturbing the pellet.
8. Resuspend cells with **500 µL** of 1X PBS and place on ice.
9. Determine the concentration of the cell suspension using cell counting equipment. For accurate cell counting, use ≥2 µL of cell suspensions and appropriate dilution factors recommended for your cell counting method.
10. (Optional) If cell suspensions appear clumpy, strain cells to a new 1.5-mL tube through a cell strainer and repeat step 9 to recount the cell suspension.
11. Transfer 1 million to 2.5 million cells to a 15-mL conical tube and place **on ice**.
12. Bring the volume of the cell suspension up to **500 µL** with 1X PBS.

Cell Fixation

Review the following table to prepare reagents before starting this section.

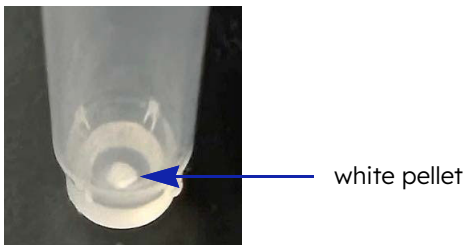
Source	Material	Take From	Place At	Brief Vortex	Brief Spin
Module A	Wash Buffer	–20°C	On ice	✗	✗
Module B	Fixation Reagent	4°C	RT	✗	✓
Other Vendors	1X PBS	—	On ice	—	—
	100% Methanol	—	On ice	—	—
	DMSO	—	RT	—	—
	DEPC	—	On ice	—	—
	Cell counting dye				
	15-mL conical tubes				
	5-mL DNA LoBind tubes				
	Chemical fume hood				

Before you Begin

- Place single-cell suspensions with 1 million to 2.5 million cells in 500 µL of 1X PBS in a 15-mL conical tube on ice.
- One Fixation Reagent tube is sufficient to fix one sample of 1 million to 2.5 million cells.
- Once thawed, invert the Wash Buffer to ensure it is fully mixed, then place on ice.
- Bring centrifuges (for 15-mL tubes) to 4°C.

Procedure

1. Once the Fixation Reagent tube has been equilibrated to **room temperature**, briefly spin down. Fixation Reagent is lyophilized at the bottom of the tube and appears as a white pellet.



2. At **room temperature** and in a chemical fume hood, add **50 µL** of DMSO to **each** Fixation Reagent tube, according to the following table.

Table 1: Reconstitution of Fixation Reagent for 1–4 samples

Reagent	1 Sample	2 Samples	4 Samples
Fixation Reagent	1 tube	2 tubes	4 tubes
DMSO	50 µL	100 µ	200 µL

3. Vortex at high speed with intermittent brief spins to dissolve the Fixation Reagent. This may take up to several minutes. Ensure that the pellet is fully dissolved before proceeding.
4. Briefly spin down the Fixation Reagent tube.
5. **On ice**, prepare Complete Fixation Solution by combining in a 5-mL DNA LoBind tube the reagents in the specified order according to Table 2.

Table 2: Complete Fixation Solution for 1 Sample

	Reagent	Volume (µL)
Step 1	Ice cold 100% methanol	2000
	Reconstituted Fixation Reagent	50
Vortex for 10 seconds. Use within 6 hours. Immediately before use, add the following:		
Step 2	DEPC	20
	Total volume	**2070**

6. Briefly vortex to mix.
7. Using the settings determined in Figure 1, vortex the cells while adding **2 mL** of the Complete Fixation Solution **dropwise** to the 15-mL conical tube containing cells.

CAUTION: Adding the fixative too quickly can result in cell clumping/incomplete fixation.

8. Incubate **on ice** for **15 minutes**.
9. Vortex the cells while adding **5 mL** of Wash Buffer **dropwise** to the tube.
10. Centrifuge the tube at **500 x g** for **5 minutes** at **4°C**.

NOTE: Nuclei or small cells can be centrifuged for 8–10 minutes to maximize recovery.

11. Carefully remove the supernatant without disturbing the pellet, leaving ~100 µL of residual volume.
12. Gently flick the tube until the cell pellet is fully resuspended in the residual volume.
13. Using a P1000 wide-bore pipette tip, add 1 mL of Wash Buffer while rinsing down the sides of the tube.
14. Transfer the fixed cells to a new 1.5-mL DNA LoBind tube.
15. Centrifuge the tube at **500 x g** for **5 minutes** at **4°C**.

16. Carefully remove the supernatant without disturbing the pellet, leaving **~50 µL** of residual volume, as shown.

17. Gently flick the tube until the cell pellet is fully resuspended in the residual volume.
18. Add **100 µL** of Wash Buffer for a total combined volume of **~150 µL**.
19. Determine the concentration of the cell suspension using cell counting equipment. For accurate cell counting, use ≥2 µL of cell suspensions and appropriate dilution factors recommended for your cell counting method.
20. Store the fixed cells, or proceed to the ScaleBio Single Cell RNA Sequencing Kit protocol.

Safe stopping point. The fixed cell suspension can be stored at −80°C for up to 12 months before proceeding with the ScaleBio Single Cell RNA Sequencing Kit protocol.

Revision History

Document, Revision	Revisions Date	Description of Change
1020802 Rev E	May 2025	Updated for QuantumScale
1020802 Rev D	July 2024	Clarified plastics used in the workflow
1020802 Rev C	April 2024	Initial release