Sample Fixation with ScalePlex – Small

Protocol

Legal Notices

Document 1087778 Rev B, May 2025

Scale Biosciences
5601 Oberlin Dr., Suite 110
San Diego, CA 92121, United States
www.scale.bio
support@scale.bio

Disclaimer of Warranty

TO THE EXTENT PERMITTED BY APPLICABLE LAW, SCALEBIO PROVIDES THIS DOCUMENT "AS IS" WITHOUT WARRANTY OF ANY KIND, INCLUDING WITHOUT LIMITATION, ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NONINFRINGEMENT. IN NO EVENT WILL SCALEBIO BE LIABLE TO YOU OR ANY THIRD PARTY FOR ANY LOSS OR DAMAGE, DIRECT OR INDIRECT, FROM THE USE OF THIS DOCUMENT, INCLUDING WITHOUT LIMITATION, LOST PROFITS, LOST INVESTMENT, BUSINESS INTERRUPTION, GOODWILL, OR LOST DATA, EVEN IF SCALEBIO IS EXPRESSLY ADVISED IN ADVANCE OF THE POSSIBILITY OF SUCH LOSS OR DAMAGE. Any warranties applicable to the ScaleBio products are set forth in the Terms and Conditions accompanying such product and such Terms and Conditions are not modified in any way by the terms of this notice.

Trademark Information

ScaleBio may make reference to products or services provided by other companies using their brand names or company names solely for the purpose of clarity, and does not assert any ownership rights over those third-party marks or names. Images were created with BioRender.com

Patent Information

ScaleBio products may be covered by one or more patents as indicated at https://scale.bio/legal-notice/.

Terms and Conditions

The use of the ScaleBio products described herein is subject to ScaleBio's Terms and Conditions that accompany the product, or such other terms as have been agreed to in writing between ScaleBio and the user.

Intended Use

All products and services described herein are intended FOR RESEARCH USE ONLY and NOT FOR USE IN DIAGNOSTIC PROCEDURES.

Table of Contents

Required Materials

Consumables and Reagents Manufactured by ScaleBio

Table 1: QuantumScale Single Cell RNA: ScalePlex Fixation Kit v2 (PN 1269724) 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
ScalePlex Oligo Module v2 (PN 1262706)	ScalePlex Oligo Plate v2	1262713	1	–20°C

Table 2: Single Cell RNA Sequencing Kit v1.1: ScalePlex Fixation Kit (PN 1072042) 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
ScalePlex Oligo Module (PN 1064645)	ScalePlex Oligo Plate	1064662	1	–20°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
5-mL DNA LoBind tubes[b]	Eppendorf	0030108310
1.5-mL DNA LoBind tubes[b]	Eppendorf	0030108418
96-well LoBind semi-skirted PCR plates[b]	Eppendorf	0030129504
Microseal ‘B’ PCR Plate Sealing Film	Bio-Rad	MSB1001
Cell strainer (optional)[c]	VWR	10032-802

Consumable or Reagent	Supplier	Part Number
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 (5-, 15-, and 50-mL tubes)	Various	Various
Vortex Mixer	Various	Various
Pipettes (P1000, P200, P20, P10)	Various	Various
Pipette controller	Various	Various
Serological pipettes (5, 10, and 25 mL)	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/nuclei 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 ScalePlex Fixation Kit is compatible with cells and nuclei for the Scale Bio Single Cell RNA Sequencing Kit v1.1 and the Scale Bio Single Cell Guide Enrichment Kit v1.1.

The ScalePlex Fixation Kit v2 is compatible with cells and nuclei for all configurations of the QuantumScale Single Cell RNA Sequencing Kits.

The ScaleBio ScalePlex Fixation Protocol is split into a Small and Large workflow depending on the desired number of samples to be processed:

- Small workflow for 8–12 samples
- Large workflow for 13–96 samples

This protocol is written specifically for fixing 8–12 samples with ScalePlex. When fixing 13–96 samples with ScalePlex, a separate protocol is provided (1122828).

This protocol shows the steps for fixation of 8–12 samples between 50,000 to 200,000 cells or nuclei with an oligo tag. The oligo tag allows multiple samples to be pooled prior to washing, which has two benefits:

1. It makes handling of low cell or nuclei numbers easier and reduces sample loss.
2. It allows for easy fixation, pooling, and counting of samples. Samples can be pooled to make one representative sample, which requires a single count before loading into the RT Plate.

We recommend performing pilot fixations to evaluate the recovery of samples with low input using this protocol. Fixation can be performed at different timepoints, and samples can be stored for up to 2 months at –80°C prior to use.

Do not mix samples containing significantly different RNA content levels in the same ScalePlex fixation. Mixing samples with disproportionate RNA levels will have results skewed towards samples containing higher RNA content.

Consumable Go-To Checklist

ScalePlex allows for the fixation of 8–96 samples simultaneously per run. Because of the range of sample inputs, specific consumables needed will vary during fixation and post-fixation washes.

Review the following table to determine which consumables are needed depending on the number of samples going into ScalePlex fixation. Consumables required for fixation of 8–12 samples are highlighted below. Refer to Required Materials on page 4 for specific vendors and lot numbers.

Note that this protocol provides instructions for fixing 8–12 samples. For instructions on fixing 13–96 samples, refer to protocol 1122828.

	Consumable	Number of Samples				
		8	9–12	13–24	25–84	85–96
Complete Fixation Solution	5-mL tube	✓	✓	—	—	—
	15-mL conical	—	—	✓	✓	—
	50-mL conical	—	—	—	—	✓
Post-Fixation Washes	5-mL tube	✓	✓	✓	✓	✓
	15-mL conical	—	—	✓	—	—
	50-mL conical	—	—	—	✓	✓
	100-mL reservoir	—	—	✓	✓	✓

Cell Preparation

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

Source	Material
Other vendors	• 1X PBS (without calcium or magnesium) • Eppendorf twin.tec LoBind PCR plates • Pipette tips (nuclease-free, filtered, wide bore for P1000) • Serological pipettes (5-, 10-, and 25-mL) • Cell counting dye • Cell counter • Cell strainer (optional)

IMPORTANT: To maintain sample quality while preparing cell/nuclei suspensions, use wide-bore pipette tips and gentle pipette mixing. Cells/nuclei must be washed with 1X PBS and resuspended in a maximum volume of 25 µL prior to fixation.

Before you Begin

- Place 1X PBS on ice.
- Bring centrifuges (for tubes or 96 well plates) to 4°C.
- Assume cell/nuclei loss throughout the process.

Procedure

1. Obtain cells/nuclei from culture, tissue dissociation, or thaw if frozen, and place **on ice**.
2. Transfer the cell/nuclei suspension into tubes or plates, depending on the number of samples and user preference.
3. Centrifuge the cells/nuclei at **500 x g** for **5 minutes** at **4°C**.

   NOTE: Nuclei and small cells can be centrifuged for 8–10 minutes to maximize recovery.
4. Carefully remove the supernatant by pipetting without disturbing the pellet.

   CAUTION: Decanting or vacuum aspirating the supernatant could lead to increased cell/nuclei loss.
5. Dilute cells/nuclei with ice-cold 1X PBS to the required target concentration (see the following table) in a **total volume of 25 µL**.
6. Resuspend the cells/nuclei by flicking. Proceed immediately to ScalePlex Fixation (for 8–12 Samples) on page 10.

   NOTE: If you would like to fix more than 12 samples, please refer to document 1122828.

ScalePlex Fixation (for 8–12 Samples)

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	✗	✓
ScalePlex Oligo Module, or ScalePlex Oligo Module v2	ScalePlex Oligo Plate, or ScalePlex Oligo Plate v2	–20°C	On ice	✗	✓
Other Vendors	1X PBS	—	On ice	—	—
	100% Methanol	—	On ice	—	—
	DMSO	—	RT	—	—
	DEPC	—	On ice	—	—
	Cell counting dye				
	Pipette tips (nuclease-free, filtered, low-retention)				
	5-mL DNA LoBind tubes				
	Chemical fume hood				
	Eppendorf twin.tec LoBind PCR plates				

Before you Begin

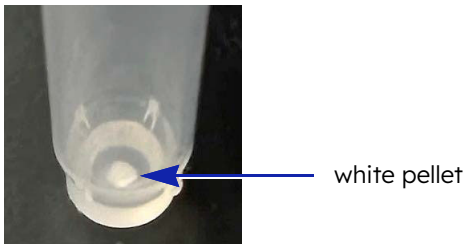
- Single-cell/nuclei suspensions with at least 25 µL of each sample in 1X PBS on ice.
- Once thawed, invert the Wash Buffer to ensure it is fully mixed, then place on ice.
- Remove the ScalePlex Oligo Plate from –20°C and place on ice.
- Bring centrifuges (for 5-mL tubes) to 4°C.

Procedure

1. Transfer **25 µL** of diluted cells/nuclei from the final step of the Cell Preparation section to a 96 well plate. Place on ice until step 9.

> **CAUTION:** Use Eppendorf twin.tec PCR LoBind plates as they demonstrated compatibility with ScalePlex fixation. The use of other PCR plates or plastics may impact data quality.

2. 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.



3. At room temperature and in a chemical fume hood, add **50 µL** DMSO to **one** Fixation Reagent tube.
4. Vortex at high speed with intermittent brief spins to dissolve the Fixation Reagent. This may take up to several minutes. Ensure the pellet is fully dissolved before proceeding.
5. Briefly spin down the Fixation Reagent tube.
6. **On ice**, prepare Complete Fixation Solution by combining the reagents (in a 5-mL DNA LoBind tube) in the specified order according to Table 3.

Table 3 – Complete Fixation Solution Preparation for one Fixation Reagent tube

	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	**2070**

NOTE: If fixing and pooling less than 8 samples, please contact your local Field Application Scientist or support@scale.bio.

7. Briefly vortex the Complete Fixation Solution to mix.
8. Spin down the ScalePlex Oligo Plate at **1000 x g** for **1 minute** at **4°C** and place **on ice**.
9. Using a sharp tool, remove the seal from the appropriate number of ScalePlex Oligo wells to be used. Transfer **5 µL** of oligos from the ScalePlex Oligo Plate to each well of diluted cells/nuclei from step 1.

NOTE: Write down the ScalePlex Oligo used for each sample to demultiplex samples during downstream data analysis.

NOTE: If the whole seal is removed, reseal unused ScalePlex Oligo Wells using a new Bio-Rad Microseal 'B' PCR Plate Sealing Film.

10. Gently pipette up and down five times to mix using a multi- or single-channel pipettor set to 25 µL.

CAUTION: Change pipette tips between wells to avoid cross-contamination. Using a pipettor set to less than 25 µL may lead to incomplete conjugation of the ScalePlex Oligo to the sample.

NOTE: Use low-retention pipette tips to limit cell loss.

11. Incubate samples **on ice** for **5 minutes**.
12. Seal the ScalePlex Oligo Plate containing unused oligos and store at –20°C.
13. Add **120 µL** of the Complete Fixation Solution **slowly** to each well containing the ScalePlex stained cells/nuclei. Gently pipette up and down five times to mix.

CAUTION: Change pipette tips between wells to avoid cross-contamination.

NOTE: Use low-retention pipette tips to limit cell or nuclei loss.

14. Incubate **on ice** for **15 minutes**.
15. Add **2.4 mL** of Wash Buffer to a 5-mL tube (on ice).
16. Use a single-channel pipette to pool samples by transferring the cell/nuclei suspension to the appropriate tube containing Wash Buffer.
17. Centrifuge the tube containing samples in Wash Buffer at **500 x g** for **5 minutes** at **4°C**.
18. Carefully remove supernatant without disturbing the pellet, leaving ~200 µL of residual volume.
19. Resuspend the cells/nuclei by flicking the tube, then add **800 µL** ice-cold Wash Buffer to the cells/nuclei.
20. Transfer all cells/nuclei (~1000 µL) to a new 5-mL DNA LoBind tube and then add **1 mL** of ice-cold Wash Buffer to the cells/nuclei.
21. Centrifuge the tube at **500 x g** for **5 minutes** at **4°C**.
22. Carefully remove the supernatant without disturbing the pellet, leaving ~50 µL of residual volume.
23. Resuspend the cells/nuclei by flicking the tube, then add **1 mL** ice-cold Wash Buffer to the cells/nuclei.
24. Centrifuge the tube at **500 x g** for **5 minutes** at **4°C**.
25. Carefully remove the supernatant without disturbing the pellet, leaving ~50 µL of residual volume.
26. Resuspend the cells/nuclei by flicking the tube, then gently add the appropriate volume of ice-cold Wash Buffer.

	8 Samples	9–12 Samples
Volume of Wash Buffer for resuspension	30 µL	50 µL

27. Determine the concentration of the cell/nuclei suspension using cell counting equipment. For accurate cell counting, use ≥2 µL of cell/nuclei suspensions and appropriate dilution factors recommended for your cell counting method.

28. Store the fixed cells/nuclei, or proceed immediately to library preparation (Single Cell RNA Sequencing, Single Cell CRISPR Guide Enrichment, or QuantumScale Single Cell RNA).

Safe stopping point. The fixed cell/nuclei suspension can be stored at –80°C for up to 2 months. Freeze cell/nuclei suspensions at a concentration between 3,000–10,000 cells/µL.

Appendix: Cell Counting Concentrations

Target Cell Input per Sample for Fixation	Target Concentration	Volume Required for Fixation
50,000	2,000 cells/µL	25 µL
100,000	4,000 cells/µL	25 µL
200,000	8,000 cells/µL	25 µL

(Optional) Determine the concentration of the cell/nuclei 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.

Although counting is optional, ensure that each sample is within the range of 50,000–200,000 cells/nuclei per sample for the highest fixation efficiency. Count cells/nuclei if you suspect that a sample may have than 200,000 cells or nuclei per sample, as this may impact fixation efficiency. Performing ScalePlex Fixation with an uneven number of cells/nuclei per sample will be represented as such in the final library, but will not impact data quality.

CAUTION: Proceeding with ScalePlex with low quality samples can result in poor quality metrics in the downstream RNA or CRISPR workflows.

CAUTION: Do not mix samples containing significantly different RNA content levels in the same ScalePlex fixation. Mixing samples with disproportionate RNA levels will have results skewed towards samples containing higher RNA content.

Revision History

Document Revision	Revisions Date	Description of Change
1087778 Rev B	May 2025	Update for ScalePlex v2
1087778 Rev A	Sept 2024	Initial release