

# **Sample Fixation with ScalePlex – Large**

## **Protocol**

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## 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%) <sup>a</sup>	Millipore Sigma	D5758-25ML
DMSO (anhydrous) <sup>a</sup>	Thermo Fisher	D12345
Methanol (purity ≥99.9%) <sup>a</sup>	Fisher Scientific	A412-500
Pipette tips (nuclease-free, filtered, low retention for P1000, P200, P20) <sup>b</sup>	Various	Various
Pipette tips (nuclease-free, filtered, wide bore for P1000) <sup>b</sup>	Various	Various
50-mL conical tubes <sup>b</sup>	Various	Various
15-mL conical tubes <sup>b</sup>	VWR	10025-286
5-mL DNA LoBind tubes <sup>b</sup>	Eppendorf	0030108310
1.5-mL DNA LoBind tubes <sup>b</sup>	Eppendorf	0030108418
Reservoir (100-mL) <sup>b</sup>	Various	Various
96-well LoBind semi-skirted PCR plates <sup>b</sup>	Eppendorf	0030129504

Consumable or Reagent	Supplier	Part Number
Microseal 'B' PCR Plate Sealing Film	Bio-Rad	MSB1001
Cell strainer (optional) <sup>c</sup>	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 (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 13–96 samples with ScalePlex. When fixing 8–12 samples with ScalePlex, a separate protocol is provided (1087778).

This protocol shows the steps for fixation of 13–96 samples between 50,000 to 200,000 cells or nuclei with an oligo tag, or as low as 10,000 cells or nuclei for sample pools of 96 samples. 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. Up to 96 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  $-80^{\circ}\text{C}$  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. Refer to [Required Materials on page 4](#) for specific vendors and lot numbers.

Note that this protocol provides instructions for fixing 13–96 samples. For instructions on fixing 8–12 samples, refer to protocol 1087778.

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  $\mu$ L prior to fixation.

## Before you Begin

- Place 1X PBS on ice.
- Bring centrifuges (for strip 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 for a **total volume of 25  $\mu$ L**.



**NOTE:** 25  $\mu$ L is essential for the proper proportion of reagents in the fixation reaction.

6. Resuspend the cells/nuclei by flicking. Proceed immediately to [ScalePlex Fixation \(for 13–96 Samples\) on page 11](#).
7. Refer to the appendix for optional counting steps. Count cells/nuclei if you suspect that a sample may have more 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.



**NOTE:** For low input samples (around 10,000 cells/nuclei per sample), ensure that most or all of the ScalePlex wells are used. This ensures sufficient cell/nuclei quantity and mitigates the risk of cell/nuclei loss during fixation and washing.

## ScalePlex Fixation (for 13–96 Samples)

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

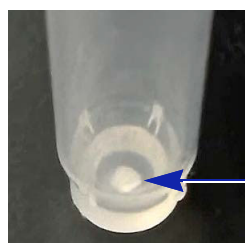
Source	Material	Take From	Place At	Brief Vortex	Brief Spin
Module A	Wash Buffer	–20°C	On ice	✗	✗
Module B	Fixation Reagent	4°C	RT	✗	✓
ScalePlex Oligo Module, or ScalePlex Oligo Module v2	ScalePlex Oligo Plate, or ScalePlex Oligo Plate v2	–20°C	On ice	✗	✓
Other Vendors	1X PBS	—	On ice	—	—
	100% Methanol	—	On ice	—	—
	DMSO	—	RT	—	—
	DEPC	—	On ice	—	—
	Cell counting dye				
	Pipette tips (nuclease-free, filtered, low-retention)				
	15-mL conical tubes				
	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  $\mu$ L 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 15-mL or 50-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.



white pellet

2. At room temperature and in a chemical fume hood, add **50  $\mu$ L** DMSO to **each** Fixation Reagent tube according to [Table 3](#).

- 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.
- Briefly spin down the Fixation Reagent tube.
- On ice**, prepare Complete Fixation Solution by combining the reagents (in a 5-mL DNA LoBind tube or 15-mL/50-mL conical tube) in the specified order according to [Table 3](#).

**Table 3 – Complete Fixation Solution Preparation**

	Reagent	13–24	25–36	37–48	49–60	61–72	73–84	85–96
<b>Step 1</b>	Ice-cold 100% methanol	4 mL	6 mL	8 mL	10 mL	12 mL	14 mL	16 mL
	Reconstituted Fixation Reagent	2 tubes 100 µL	3 tubes 150 µL	4 tubes 200 µL	5 tubes 250 µL	6 tubes 300 µL	7 tubes 350 µL	8 tubes 400 µL
Vortex for 10 seconds. Use within 6 hours. Immediately before use, add the following:								
<b>Step 2</b>	DEPC	40 µL	60 µL	80 µL	100 µL	120 µL	140 µL	160 µL
	<b>Total (mL)</b>	<b>4.14</b>	<b>6.21</b>	<b>8.28</b>	<b>10.35</b>	<b>12.42</b>	<b>14.49</b>	<b>16.56</b>

- Briefly vortex the Complete Fixation Solution to mix.
- Spin down the ScalePlex Oligo Plate at **1000 x g** for **1 minute** at **4°C**. Remove the seal and place **on ice**.
- Aliquot **25 µL** of the cell/nuclei suspensions to each corresponding sample well of the ScalePlex Oligo Plate. Gently pipette up and down five times to mix.



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



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



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

- Incubate samples **on ice** for **5 minutes**.
- Pour Complete Fixation Solution into a 100-mL reservoir.
- Add **120 µL** of the Complete Fixation Solution **slowly** to each well of the plate 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.

- Incubate **on ice** for **15 minutes**.

13. Place a 100-mL reservoir on ice and add the following volume of Wash Buffer to the reservoir.

	13–24 Samples	25–48 Samples	49–96 Samples
Volume of Wash Buffer for first wash	7 mL	15 mL	29 mL

14. Use a multi-channel pipette to pool samples by transferring the cell/nuclei suspensions from the ScalePlex Oligo Plate to the reservoir containing Wash Buffer. Gently pipette the cell/nuclei suspension and transfer the cells/nuclei with Wash Buffer to either a 15-mL conical tube (13–24 samples) or 50-mL conical tube (>25 samples).
15. If unused ScalePlex Oligos remain, seal the ScalePlex Oligo Plate and store at **–20°C**.
16. Add **4 mL** of ice-cold Wash Buffer to the now empty 100-mL reservoir, rinsing the sides of the reservoir 2–3 times to collect the residual liquid in the reservoir. Leave the Wash Buffer in the reservoir **on ice**. This volume will be used in [step 21](#).
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.
21. Collect the Wash Buffer from the 100-mL reservoir and add it to the cell/nuclei suspension.
22. Centrifuge the tube at **500 x g** for **5 minutes** at **4°C**.
23. Carefully remove the supernatant without disturbing the pellet, leaving ~50 µL of residual volume.
24. Resuspend the cells/nuclei by flicking the tube, then add **1 mL** ice-cold Wash Buffer to the cells/nuclei.
25. Centrifuge the tube at **500 x g** for **5 minutes** at **4°C**.
26. Carefully remove the supernatant without disturbing the pellet, leaving ~50 µL of residual volume.
27. Resuspend the cells/nuclei by flicking the tube, then gently add the appropriate volume of ice-cold Wash Buffer.

	13–24 Samples	25–48 Samples	49–96 Samples
Volume of Wash Buffer for resuspension	100 µL	100 µL	200 µL

28. 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.
29. 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



**NOTE:** For low input samples (around 10,000 cells/nuclei per sample), ensure that most or all of the ScalePlex wells are used. This ensures sufficient cell/nuclei quantity and mitigates the risk of cell/nuclei loss during fixation and washing.

Target Cell Input per Sample for Fixation	Target Concentration	Volume Required for Fixation
10,000	400 cells/ $\mu$ L	25 $\mu$ L
50,000	2,000 cells/ $\mu$ L	25 $\mu$ L
100,000	4,000 cells/ $\mu$ L	25 $\mu$ L
200,000	8,000 cells/ $\mu$ L	25 $\mu$ L

(Optional) Determine the concentration of the cell/nuclei suspension using cell counting equipment. For accurate cell counting, use  $\geq 2$   $\mu$ L of cell/nuclei suspensions and appropriate dilution factors recommended for your cell counting method.

Although counting is optional, ensure that each sample is below 200,000 cells/nuclei per sample for the highest fixation efficiency. Count cells/nuclei if you suspect that a sample may have more 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
1122828 Rev C	May 2025	Update for ScalePlex v2
1122828 Rev B	Sept 2024	Update to Large Protocol
1122828 Rev A	June 2024	Initial release