Scale FixKit

Cell Fixation

Laboratory Protocol

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Introduction

The ScaleBio FixKit Cell Fixation protocol is intended for fixing cells prior to use in ScaleBio's library preparation protocols. Fixation can be performed at different timepoints, as samples can be stored for up to 1 month at -80°C prior to use, reducing batch effects during library preparation.

Required Materials

Consumables and reagents manufactured by Scale Biosciences Scale FixKit:

Consumable	Quantity	Part Number	Storage Temp
Fixation Reagent 1	12		
WRS Buffer	12		

Consumables and reagents manufactured by other vendors:

Consumable or Reagent	Supplier	Part Number	Storage Temp
1X PBS without calcium or magnesium	Various	Various	RT
DEPC	Millipore Sigma	D5758-25ML	
DMSO	Thermo	D12345	
Methanol	Fisher Scientific	A412-500	
Sterile, filtered, low retention tips for P1000, P200, P20, P10 pipettes	Various	Various	
Sterile, filtered, wide bore tips for P1000, P200 pipettes	Various	Various	
15-mL conical tubes	VWR	10025-286	
50-mL conical tubes	Falcon	352070	
1.5 mL LoBind Eppendorf tubes	Eppendorf	0030108418	
Flowmi 40 µm filters	VWR	10032-802	
Cell counting dye: AO/PI, Trypan Blue, YOYO-1, etc.	Various	Various	

Required Equipment:

Item	Supplier	Part Number
Temperature-controlled centrifuges (for 15-mL & 1.5-mL tubes)	Various	
Vortex Mixer	Scientific Industries	SI-0236
P1000 pipette	Various	
P200 pipette	Various	
P20 pipette	Various	
P10 pipette	Various	
Cell counting instrument: Hemocytometer, Nexcelom, Cellometer K2, etc.	Various	
Cell counting slides	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.
- Unless otherwise specified, thaw reagents on ice.
- Unless otherwise specified, vortex reagents.
- Handle Fixation Reagent 1, DEPC, and Methanol in a chemical fume hood.
- Never reuse pipette tips or tubes.
- Use wide-bore tips for pipetting cell mixtures.
- 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.
- Keep pipette tip boxes, reagent containers, and sample tubes closed when not in use.
- Routinely wipe work surfaces with a 10% bleach solution.

Before you begin:

- Bring tubes of Fixation Reagent 1 to room temperature.
- Fully chill 100% Methanol on ice.

Note: Centrifugation of 15-mL conical tubes and 1.5-mL microcentrifuge tubes are performed at 4°C. Bring centrifuges that accommodate these two tube formats to 4°C.

- Place 1X PBS and WRS buffer on ice.
- 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 liquid reaches the 5 mL mark on a 15-mL conical tube as shown below:

500 µl water without vortexing

500 µl water with vortexing

Buffer Preparation

Important: Prepare buffers using a vortex mixer in a chemical fume hood.

Materials:

- Fixation Reagent (1 tube per sample)
- DMSO (50 µl per sample)
- Ice-cold 100% Methanol (2 mL per sample)
- 15-mL conical tube (1 tube per sample)

Procedure:

1. Prepare Complete Cell Fixation Solution
    a. **In a chemical fume hood**, reconstitute Fixation Reagent 1 by adding 50 µl DMSO to each tube. Fixation Reagent 1 is lyophilized at the bottom of the tube and appears as a white pellet.

    b. Dissolve tube contents by repeatedly vortexing at high speed with intermittent brief spins to bring contents to the bottom of the tube. Repeat this process until all solids are fully dissolved; this may take up to several minutes. Ensure that all solids are fully dissolved before proceeding.
    c. Briefly spin down the Fixation Reagent 1 tube to bring contents to the bottom of the tube.
    d. Prepare Complete Cell Fixation solution by combining ice-cold 100% methanol with reconstituted Fixation Reagent 1 in a conical tube according to the volumes provided in the table below:

Reagent	1 Sample	2 Samples	4 Samples
100% Methanol	2 mL	4 mL	8 mL
Reconstituted Fixation Reagent 1	50 µl	100 µl	200 µl

    e. Vortex for 10 seconds to mix and place on ice. Use within 6 hours.

Cell Preparation

Important: We recommend use of wide-bore pipette tips, and gentle pipette-mixing, for preparing cell suspensions to maintain sample quality. A total of 2.5 million or fewer cells can be processed per tube of Fixation Reagent 1. Cells must be washed out of their preparation medium with 1x PBS without calcium or magnesium and resuspended in a maximum volume of 500 µL prior to fixation.

Materials:

- Ice-cold 1X PBS without calcium or magnesium
- Sterile, filtered, wide-bore pipette tips
- 15-mL conical tubes
- 40 µm Flowmi Cell Strainers
- Cell counting dye (Trypan Blue, YOYO-1, AO/PI, etc.) and required instrumentation (hemocytometer, Countess, Nexcelom Cellometer K2, etc.)
- Counting slides

Procedure:

1. Obtain cells from culture or prepare from frozen.
2. Wash cells with ice-cold 1X PBS.
3. Centrifuge for 500 x g for 5 min at 4°C.
4. Remove supernatant taking care to not aspirate the cell pellet.
5. Resuspend cells with 500 µL 1X PBS.
6. Strain cells to a new 1.5-mL tube through 40 µm Flowmi cell strainer.
7. Determine cell concentration using a viability dye and a hemocytometer, Nexcelom Cellometer K2, or similar cell counting equipment. For accurate cell counting, use >=2 µl of cell suspensions and appropriate dilution factors to obtain cell counts that are recommended for accuracy for your cell counting method.
8. Transfer 2.5 million or fewer cells to a 15-mL conical tube and place on ice.
9. Bring the volume of the cell suspension up to 500 µl with 1X PBS.

Cell Fixation

Duration: 45 minutes

Materials:

- Flowmi strained and counted single cell suspensions in a 15-mL conical tube
- DEPC (20 µl per sample)
- Complete Cell Fixation Solution (2 mL per sample)
- WRS Buffer (6.5 mL per sample)
- Sterile, filtered, wide-bore P1000 pipette tips

Note: All steps below are done on ice with centrifugations done at 4°C.

Procedure:

1. Immediately before use, add 20 µl of DEPC per 2 mL of the Complete Cell Fixation Solution (prepared on page 5, step d). Briefly vortex to mix.
2. Using the settings determined previously, vortex the cells while adding 2 mL of the Complete Cell Fixation Solution + DEPC dropwise to the tube.

Caution: Adding the fixative to quickly to the cells can result in cell clumping/incomplete fixation.

3. Incubate the tube on ice for 15 minutes.
4. Vortex the cells while adding 5 mL of WRS Buffer dropwise to the tube.
5. Centrifuge the tube at 500 x g for 5 min at 4°C.
6. Carefully remove supernatant without disturbing the pellet, leaving ~50 µl of residual volume as shown:

7. Gently flick the cell pellet until the cells are fully resuspended in the residual volume in the tube.
8. Using a wide-bore pipette tip, add 1 mL of WRS Buffer while rinsing down the sides of the tube and transfer the fixed cells to a new 1.5-mL tube.
9. Centrifuge the tube for 500 x g for 5 min at 4°C.
10. Carefully remove supernatant without disturbing the pellet, leaving ~50 µl of residual volume.
11. Gently flick the cell pellet until the cells are fully resuspended in the residual volume in the tube.
12. Add 100 µl of WRS Buffer and gently flick the tube until cells are fully resuspended.
13. Determine cell concentration using a viability dye and a hemocytometer, Nexcelom Cellometer K2, or similar cell counting equipment. For accurate cell counting, use >=2 µl of cell suspensions and appropriate dilution factors to obtain cell counts that are recommended for accuracy for your cell counting method.

Note: Fixed cells may settle at the bottom of the tube. To ensure even distribution of cells, flick the tube 10-15 times until pellet has dispersed before counting cell suspensions.

14. If fixed cells will not be used within the same day as preparation, freeze cells at -80°C.

Frozen cells can be stored at -80°C for up to 1 month prior to use.