Fixation of Cells for Chromium Fixed RNA Profiling

Introduction

Chromium Fixed RNA Profiling offers comprehensive scalable solutions to measure gene expression in single cell that are fixed with formaldehyde. This protocol outlines how to perform fixation on single cell for use with Chromium Fixed RNA Profiling workflow.

Sample Quality

- Use high quality single cell that can withstand the fixation steps.
- This fixation protocol requires ≥300,000 cells.
- Highly viable single cell (>80%) will have the greatest sensitivity and cell recovery.
- Samples should have minimal debris for best results; debris can have associated RNA that can contribute to non-cell background.
- If possible, please sort for live cells via flow cytometry or perform bead clean-up to remove dead cells prior to beginning the cell fixation protocol.

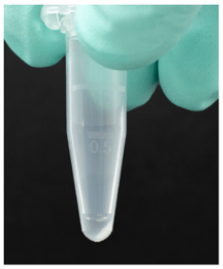
Buffer Preparation – Prepare fresh

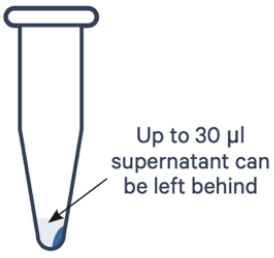
Day 1 - Fixation Buffer (Maintain at Room Temp.)	Stock	Final	Per Sample (µl)
Nuclease-free Water	-	-	792
Concentrated Fixation Buffer	10X	1X	100
Formaldehyde	37%	4%	108
Day 2 **- Quenching Buffer (Maintain at 4°C)**	**Stock**	**Final**	**Per Sample (µl)**
Nuclease-free Water	-	-	875
Concentrated Quench Buffer	8X	1X	125

Cell Fixation Protocol

Day 1

a. Centrifuge sample at **300-400 rcf** for **5 min** (PBMCs/cell lines) or **150 rcf** for **10 min** (dissociated tumor cells) at **4°C**.
b. Remove the supernatant without disturbing the pellet. Up to 30 µl supernatant may be left behind to optimize cell recovery without significantly impacting assay performance.
c. Add **1 ml** Fixation Buffer to the sample pellet and pipette mix 5x.
d. Incubate for ***exactly*** **20 h** at **4°C.**
e. *DO NOT agitate or mix the sample during incubation.*





Day 2

a. Centrifuge at **850 rcf** for **5 min** at **room temperature**.
b. Remove the supernatant without disturbing the pellet.
c. Add **1 ml chilled** Quenching Buffer to the sample pellet and pipette mix 5x and keep on ice.
d. Thaw Enhancer for **10 min** at **65°C**. Vortex and centrifuge briefly. Keep warm and verify no precipitate before use.
e. *DO NOT keep the thawed reagent on ice, or the solution will precipitate. Once thawed, Enhancer can be kept at 42°C for up to 10 min.*
f. Add 100 µl pre-warmed Enhancer to 1,000 µl fixed sample in Quenching Buffer. Pipette mix.
g. Add 275 µl 50% glycerol to 1,100 µl fixed sample in Quenching Buffer and Enhancer. Pipette mix.
h. Email Nicole.Manning@CUAnschutz.edu to schedule your sample drop off.
i. Drop off your **samples** and **'Sample Information Form'** at the **Genomics Core** located at **RC2-P15-9400A**.

Genomics and Microarray Core
University of Colorado - Anschutz Medical Campus

Sample Information Form

Instructions: Please accompany this form when you drop-off your samples at the Genomics Core located at RC2-P15-9400A.

Principal Investigator: _____

Sample Drop-off Date: _____

	Sample Name	Group
1.	_____	/_____
2.	_____	/_____
3.	_____	/_____
4.	_____	/_____
5.	_____	/_____
6.	_____	/_____
7.	_____	/_____
8.	_____	/_____
9.	_____	/_____
10.	_____	/_____
11.	_____	/_____
12.	_____	/_____
13.	_____	/_____
14.	_____	/_____
15.	_____	/_____
16.	_____	/_____