

DEMONSTRATED PROTOCOL

Nuclei Isolation for Single Cell ATAC Sequencing

Overview

This protocol outlines how to isolate, wash, and count nuclei suspensions for use with the Chromium Single Cell ATAC protocol. Cryopreserved primary cells (PBMCs) and cell lines (GM12878 cells; EL4 cells) were used to develop this protocol. PBMCs were cryopreserved in IMDM + 40% FBS + 15% DMSO. Cell lines were cryopreserved in RPMI + 15% FBS + 5% DMSO. Optimization of some protocol steps (e.g. lysis time, centrifugation speed/time and filtration steps) may be needed based on cell type.



The recommended buffer compositions, final nuclei suspension concentration, and the wash step guidelines presented in this protocol for nuclei sample preparation are critical for optimal Chromium Single Cell ATAC assay performance. Failure to adhere to these guidelines may result in compromised microfluidics chip operation.

Additional Guidance

Consult Demonstrated Protocol Cell Preparation Guide (Document CG000053) for Tips & Best Practices.

Cells carry potentially hazardous pathogens. Follow material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage and disposal of biological materials.

Preparation – Buffers

Diluted Nuclei Buffer	Stock	Final	1 ml
Maintain at 4°C			
Nuclei Buffer (10x Genomics, PN-2000153)	20X	1X	50 µl
Nuclease-free Water	-	-	950 µl

Wash Buffer	Stock	Final	10 ml
Prepare fresh, maintain at 4°C			
Tris-HCl (pH 7.4)	1M	10 mM	100 µl
NaCl	5M	10 mM	20 µl
MgCl ₂	1M	3 mM	30 µl
BSA	10%	1%	1 ml
Tween-20	10%	0.1%	100 µl
Nuclease-free Water	-	-	8.75 ml

Lysis Buffer	Stock	Final	5 ml
Prepare fresh, maintain at 4°C			
Tris-HCl (pH 7.4)	1 M	10 mM	50 µl
NaCl	5 M	10 mM	10 µl
MgCl ₂	1 M	3 mM	15 µl
Tween-20	10%	0.1%	50 µl
Nonidet P40 Substitute (if using Sigma (74385) 100% solution, prepare a 10% stock)	10%	0.1%	50 µl
Digitonin (incubate at 65°C to dissolve precipitate before use)	5%	0.01%	10 µl
BSA	10%	1%	500 µl
Nuclease-free Water	-	-	4.315 ml

Additional Buffers

RPMI + 10% FBS (maintain at 4°C)

PBS + 0.04% BSA (maintain at 4°C)

Specific Reagents & Consumables

Vendor	Item	Part Number
10x Genomics	Nuclei Buffer	2000153
Thermo Fisher Scientific	Digitonin Tubes, 0.2 ml, flat cap tube Sorvall Microtube Adapters	300410 AB0620 76003750
Millipore-Sigma	Trizma Hydrochloride Solution, pH 7.4 Sodium Chloride Solution, 5M Magnesium Chloride Solution, 1M Nonidet P40 Substitute	T2194 59222C M1028 74385
Miltenyi Biotec	MACS BSA Stock Solution	130-091-376
Bel-Art	Flowmi Cell Strainer, 40 µm	H13680-0040

Protocol Overview

1. Thaw Cells

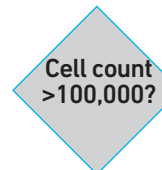
(For fresh cells proceed to Nuclei Isolation after determining cell count)



Based on cell type, thaw cells using the protocol for thawing cell lines or primary cells/fragile cells

Resuspend cell pellet
(in 1 ml PBS + 0.04% BSA)

Determine cell concentration



See Appendix for
Low Cell Input Nuclei Isolation protocol

2. Nuclei Isolation



Add cell suspension to 2-ml tube
Centrifuge (300 rcf, 5 min)

Remove Supernatant

Add Lysis Buffer to pellet
Pipette mix



Incubate on ice (3-5 min*)
*Optimize time for complete cell lysis

Add Wash Buffer
Pipette mix

Centrifuge (500 rcf, 5 min)

Remove Supernatant
DO NOT disturb pellet



Resuspend in Diluted Nuclei Buffer
Critical for optimal assay performance

Determine final nuclei concentration

Proceed to Chromium Single Cell ATAC Reagent Kits Protocol (CG000168)

Protocol

This protocol was demonstrated using commercially-sourced cells.

Cell Type	Species	Supplier
GM12878	Human	Coriell Institute
EL4	Mouse	ATCC
Normal Peripheral Blood MNC (PBMC)	Human	AllCells

1. Thaw Cells (if using frozen cells)

For cell lines (used for GM12878 and EL4 cells):

- Remove cryovial(s) from storage, thaw in the water bath at **37°C for 1–2 min**. Remove from the water bath when a small ice crystal remains in the cryovial.
- Pipette mix the cells and transfer to a 15-ml conical tube containing **10 ml** pre-warmed media (RPMI + 10% FBS).
- Centrifuge at **300 rcf for 5 min**.
- Remove the supernatant without disrupting the cell pellet and resuspend in **1 ml** PBS + 0.04% BSA. Transfer to a 2-ml microcentrifuge tube. Rinse the 15-ml tube with **0.5 ml** PBS + 0.04% BSA and transfer the rinse to the 2-ml tube containing the cells.
- Centrifuge cells at **300 rcf for 5 min**.
- Remove the supernatant without disrupting the cell pellet and resuspend in **1 ml** PBS + 0.04% BSA.
- Pass cell suspension through a **40 µm** Flowmi Cell Strainer.
- Determine the cell concentration using a Countess II FL Automated Cell Counter (see Appendix) or the hemocytometer.
- Proceed to Nuclei Isolation (step 2).
If cell count is <100,000, nuclei may be isolated using the Low Cell Input Nuclei Isolation protocol (see Appendix).


For primary cells/fragile cells (used for PBMCs):


- Remove cryovial(s) from storage, thaw in the water bath at **37°C for 1–2 min**. Remove from the water bath when a small ice crystal remains in the cryovial.
- Transfer the thawed cells to a 50-ml conical tube. Rinse the cryovial with **1 ml** pre-warmed media (RPMI + 10% FBS) and add the rinse dropwise to the 50-ml conical tube while gently shaking the tube.
- Serially dilute cells with complete growth medium a total of 5 times (including dilution at step b) by 1:1 volume additions with **~1 min** wait between additions. Add media (RPMI + 10% FBS) at a speed of **3–5 sec/ml**.
- Centrifuge at **300 rcf for 5 min**.
- Transfer most of the supernatant to a new tube, leaving **~1 ml** and resuspend the cell pellet in this volume. Save the removed supernatant until the protocol is complete.
- Add an additional **9 ml** media (at a speed of 3–5 sec/ml) to achieve a total volume of **~10 ml**.
- Centrifuge at **300 rcf for 5 min**.
- Remove the supernatant without disrupting the cell pellet and resuspend in **1 ml** PBS + 0.04% BSA. Transfer to a 2-ml microcentrifuge tube. Rinse the 50-ml tube with **0.5 ml** PBS + 0.04% BSA and transfer the rinse to the 2-ml tube containing the cells.

- Centrifuge cells at **300 rcf for 5 min**.
- Remove the supernatant without disrupting the cell pellet and resuspend in **1 ml** PBS + 0.04% BSA.
- Pass cell suspension through a **40 µm** Flowmi Cell Strainer.
- Determine the cell concentration using a Countess II FL Automated Cell Counter (see Appendix) or the hemocytometer.
- Proceed to Nuclei Isolation (step 2).
If cell count is <100,000, nuclei may be isolated using the Low Cell Input Nuclei Isolation protocol (see Appendix).

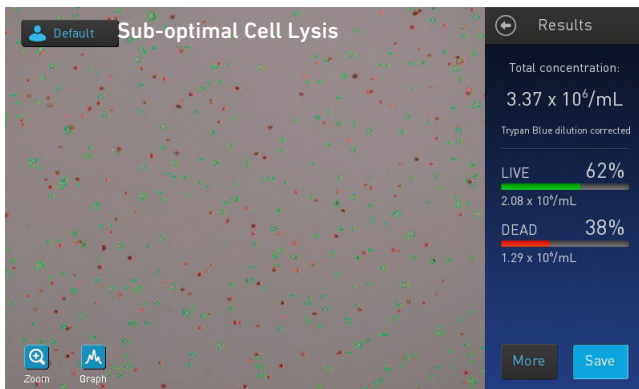
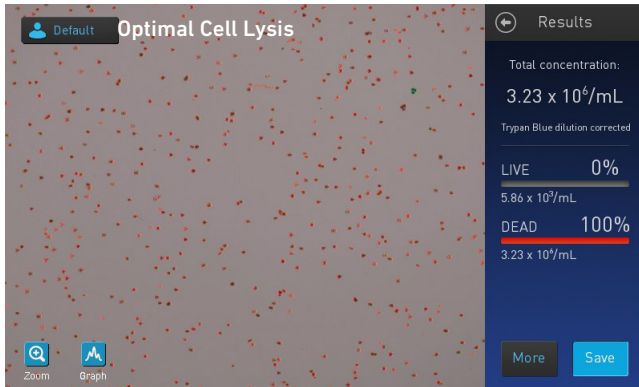
2. Nuclei Isolation

Nuclei may be isolated from 100,000–1,000,000 cells using this protocol.

- Add 100,000–1,000,000 cells to a 2-ml microcentrifuge tube. Centrifuge at **300 rcf for 5 min** at **4°C**.
 - Remove ALL the supernatant without disrupting the cell pellet.
 - Add **100 µl** chilled Lysis Buffer. Pipette mix 10x.
 - Incubate for **3–5 min*** on ice.
*Cryopreserved PBMCs were incubated for **3 min**
*Cryopreserved cell lines were incubated for **5 min**
-  Optimize incubation time based on cell type. Sub-optimal or prolonged lysis times can both alter assay performance. Assess lysis efficacy via the Countess II FL Automated Cell Counter/microscopy. See Results for optimal cell lysis.
- Add **1 ml** chilled Wash Buffer to the lysed cells. Pipette mix 5x.
 - Centrifuge at **500 rcf for 5 min** at **4°C**.
 - Remove the supernatant without disrupting the nuclei pellet.
 - Based on nuclei concentration determined before step 2a and assuming **~50%** nuclei loss during cell lysis, resuspend in chilled Diluted Nuclei Buffer. See Nuclei Stock Concentration Table in Appendix for guidance on nuclei suspension concentration. Maintain on ice.

-  The use of the Diluted Nuclei Buffer for nuclei suspension is critical for optimal Single Cell ATAC assay performance. The composition of the Tris-based Diluted Nuclei Buffer, including Magnesium concentration, has been optimized for the Transposition and Barcoding steps in the Single Cell ATAC protocol. Suspension of nuclei in a different buffer may not be compatible with these protocol steps.
- OPTIONAL** If cell debris and large clumps are observed, pass through a cell strainer. For low volume, use a **40 µm** Flowmi Cell Strainer to minimize volume loss.
 - Determine the nuclei concentration using a Countess II FL Automated Cell Counter (see Appendix) or the hemocytometer.
 - Proceed **immediately** to Chromium Single Cell ATAC Reagent Kits protocol (CG000168).

Results



Troubleshooting

Problem	Possible Solution
High fraction of non-viable cells in input material prior to starting nuclei isolation	Optimize cell thawing to enhance sample quality
	Reduce fraction of dead cells. Refer to Demonstrated Protocol Removal of Dead Cells from Single Cell Suspensions for Single Cell RNA Sequencing (Document CG000093)
	Sort cells using flow cytometry
	Gently handle cell suspensions by following best practices and reduce cell processing times
High fraction of viable cells post cell lysis	Incrementally increase the lysis time and monitor lysis efficacy microscopically

Appendix

Nuclei Counting and Viability

Countess II FL Automated Cell Counter is recommended for determining nuclei concentrations. The optimal range of cell concentration for Cell Counter is 1,000-10,000 cells/ μ L. Refer to manufacturer's instructions for details on operations.

- Vortex 0.4% trypan blue stain, centrifuge briefly and aliquot 10 μ L per tube.
- Pipette mix the nuclei suspension. **Immediately** add 10 μ L nuclei suspension to 10 μ L aliquot of 0.4% trypan blue stain. Gently pipette mix 10x.
- Transfer 10 μ L trypan blue stained nuclei to a Countess II Cell Counting Slide chamber.
- Insert the slide into the Countess II FL Cell Counter, and determine the nuclei concentration and viability. <5% of input cells should be viable. Optimize focusing and light exposure.

Nuclei Stock Concentration Table

Based on the Targeted Nuclei Recovery, prepare the nuclei suspension in Diluted Nuclei Buffer to achieve the corresponding Nuclei Stock concentrations.

Targeted Nuclei Recovery	Nuclei Stock Concentration (nuclei/ μ L)
500	155-390
1,000	310-780
2,000	610-1,540
3,000	925-2,300
4,000	1,230-3,075
5,000	1,540-3,850
6,000	1,850-4,600
7,000	2,150-5,400
8,000	2,460-6,150
9,000	2,770-6,900
10,000	3,080-7,700

Appendix

Low Cell Input Nuclei Isolation

Nuclei may be isolated from 2,000-100,000 cells using this protocol. If cell count is <40,000, centrifuge cell suspension at **300 rcf for 5 min at 4°C** and resuspend the cell pellet in **50 µl PBS + 0.04% BSA**. Transfer **50 µl** cell suspension to a 0.2-ml tube. Proceed directly to **step c**.

- a. Centrifuge cell suspension at **300 rcf for 5 min at 4°C**. Remove supernatant and resuspend pellet in PBS + 0.04% BSA for 1,000 cells/µl cell suspension.
- b. Add 2,000–40,000 cells to a 0.2-ml tube in a total volume of **50 µl PBS + 0.04% BSA**.

Approximately 25% of the cell input is expected to be recovered during Chromium Single Cell ATAC sequencing.

Cell Input	Expected Nuclei Recovery (after cell lysis)	Expected Nuclei Recovery (ATAC sequencing)
40,000	16,000	10,000
20,000	8,000	5,000
10,000	4,000	2,500
4,000	1,600	1,000
2,000	800	500

- c. Centrifuge at **300 rcf for 5 min at 4°C**.
- d. Remove **45 µl** supernatant without touching the bottom of the tube to avoid dislodging the cell pellet.
- e. Add **45 µl** chilled Lysis Buffer. Gently pipette mix 3x.
- f. Incubate for **3-5 min*** on ice.

*Cryopreserved PBMCs were incubated for **3 min**

*Cryopreserved cell lines were incubated for **5 min**

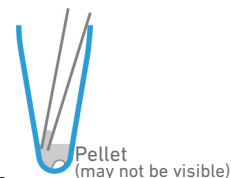


Optimize incubation time based on cell type. Sub-optimal or prolonged lysis times can both alter assay performance. Assess lysis efficacy via the Countess II FL Automated Cell Counter/microscopy. See Results for optimal cell lysis.

- g. Add **50 µl** chilled Wash Buffer to the tube. **DO NOT** mix.

- h. Centrifuge at **500 rcf for 5 min at 4°C**.
- i. Remove **95 µl** supernatant without disrupting the nuclei pellet.
- j. Add **45 µl** chilled Diluted Nuclei Buffer to the pellet. **DO NOT** mix.
- k. Centrifuge at **500 rcf for 5 min at 4°C**.

- l. Remove the supernatant without touching the bottom of the tube to avoid dislodging the nuclei pellet.



The supernatant may be removed in two steps, first with a 100-µl pipette (set to 40 µl), followed by removal with a 10-µl pipette (set to 10 µl).

- m. Resuspend the nuclei pellet in **7 µl** chilled Diluted Nuclei Buffer (pellet may not be visible).



The use of the Diluted Nuclei Buffer for nuclei suspension is critical for optimal Single Cell ATAC assay performance. The composition of the Tris-based Diluted Nuclei Buffer, including Magnesium concentration, has been optimized for the Transposition and Barcoding steps in the Single Cell ATAC protocol. Suspension of nuclei in a different buffer may not be compatible with these protocol steps.

- n. Use **2 µl** nuclei suspension to determine the cell concentration by a Countess II FL Automated Cell Counter (see Appendix) or the hemocytometer. A final nuclei concentration of **30 nuclei/µl** is needed for Targeted Nuclei Recovery of 500.
- o. Proceed **immediately** with **5 µl** nuclei suspension to Chromium Single Cell ATAC Reagent Kits protocol (CG000168).

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