

Nuclei Isolation for Single Cell Multiome ATAC + Gene Expression Sequencing

Introduction

This protocol outlines how to isolate, wash, and count nuclei suspensions for use with the **GEM-X Epi Multiome (CG001689)** and **Next GEM Multiome (CG000338) ATAC + Gene Expression (GEX)** protocols. Cryopreserved primary cells (PBMCs) and cell lines (GM12878 cells; 3T3 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 or Cryo Stor 10.

 For optimal assay performance, nuclei isolation should be performed using this protocol and not the standalone protocols for nuclei isolation for ATAC or RNA sequencing only. 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 Multiome ATAC + GEX assay performance. Failure to adhere to these guidelines may result in suboptimal assay performance.

DO NOT use this protocol for isolating nuclei from tissue-derived cells. Refer to Chromium Nuclei Isolation Kit Sample Prep User Guide (CG000505 Rev B & later revisions) & Nuclei Isolation from Complex Tissues for Single Cell Multiome ATAC + Gene Expression Sequencing (CG000375) for isolating nuclei from tissue-derived cells.

Consult Demonstrated Protocol Cell Preparation Guide (Document CG00053) for Tips & Best Practices.

Additional Guidance

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.

Cell Sourcing

Cell Type	Species	Supplier
GM12878	Human	Coriell Institute
3T3	Mouse	ATCC
Normal Peripheral Blood MNC (PBMC)	Human	AlloCells

Optimization Recommendations

The following demonstrated protocol was performed using the indicated sample types. Optimization of some protocol steps may be needed for other cell types.

- **Lysis time:** Perform a lysis timeline to determine appropriate lysis incubation time for a specific cell type.
For optimization experiments, RNase inhibitor may be omitted from the buffer and instead of the 10x Genomics' Nuclei Buffer, PBS may be used for nuclei resuspension. However for the actual experiment, ensure that RNase inhibitor and the 1x Nuclei Buffer are used as recommended.
- **Lysis buffer strength:** If nuclei quality is poor at short lysis times, buffer strength can be decreased for a gentler lysis
- **Sample cleanup steps:** Additional cleanup steps such as washes, filtering, density gradient centrifugation, and FACS may be necessary to clean up excess debris present in the sample

See Appendix for additional optimization and troubleshooting guidance.

Preparation – Buffers

Diluted Nuclei Buffer	Stock	Final	1 ml
<i>Prepare fresh, maintain at 4°C</i>			
Nuclei Buffer* (20X)	20X	1X	50 µl
DTT	1000 mM	1mM	1 µl
RNase Inhibitor (2001488 or equivalent)	40X	1X	25 µl
Nuclease-free Water	-	-	924 µl

Wash Buffer	Stock	Final	4 ml
<i>Prepare fresh, maintain at 4°C</i>			
Tris-HCl (pH 7.4)	1 M	10 mM	40 µl
NaCl	5 M	10 mM	8 µl
MgCl ₂	1 M	3 mM	12 µl
BSA	10%	1%	400 µl
Tween-20	10%	0.1%	40 µl
DTT	1000 mM	1 mM	4 µl
RNase Inhibitor (2001488 or equivalent)	40X	1X	100 µl
Nuclease-free Water	-	-	3.40 ml

Lysis Buffer	Stock	Final	2 ml
<i>Prepare fresh, maintain at 4°C</i>			
Tris-HCl (pH 7.4)	1 M	10 mM	20 µl
NaCl	5 M	10 mM	4 µl
MgCl ₂	1 M	3 mM	6 µl
Tween-20	10%	0.1%	20 µl
Nonidet P40 Substitute (alternatively, use IGEAL CA-630)	10%	0.1%	20 µl
If using Sigma 74385 or i8896, prepare a 10% stock			
Digitonin (incubate at 65°C to dissolve precipitate before use)	5%	0.01%	4 µl
BSA	10%	1%	200 µl
DTT	1000 mM	1 mM	2 µl
RNase Inhibitor (2001488 or equivalent)	40X	1X	50 µl
Nuclease-free Water	-	-	1.67 ml

Additional Buffers

RPMI + 10% FBS (maintain at 4°C, pre-warm at 37°C before use)

PBS + 0.04% BSA (maintain at 4°C)

Specific Reagents & Consumables

Vendor	Item	Part Number
10x Genomics	Nuclei Buffer* (20X)	2000153 / 2000207
	RNase Inhibitor 40X	2001488
Thermo Fisher	Digitonin	BN2006
	Tubes, 0.2 ml, flat cap tube**	AB0620
Fisher Scientific	Sorvall Microtube Adapters**	76003750
Sigma-Aldrich	Trizma Hydrochloride Solution, pH 7.4	T2194
	Sodium Chloride Solution, 5 M	59222C
	Magnesium Chloride Solution, 1M	M1028
	Nonidet P40 Substitute	74385
	(alternatively, use IGEAL CA-630)	i8896
	Protector RNase inhibitor (alternative to 10x Genomics product)	3335402001
	DTT	646563
Miltenyi Biotec	MACS BSA Stock Solution	130-091-376
Bel-Art	Flowmi Cell Strainer, 40 µm	H13680-0040
Bio-Rad	Tween 20	1662404
Corning	1X Phosphate-Buffered Saline, pH 7.4	21-040-CV
Optional <i>Needed only if removing granulocytes</i>		
Stemcell Technologies	EasySep™ Direct Human PBMC Isolation Kit	19654

*Included in 10x Genomics kits. Depending on the downstream workflow, refer to applicable user guides for kit details.

**ONLY for Low Cell Input Nuclei Isolation protocol

See Appendix for DNase Treatment specific reagents & buffers

Specific Reagents & Consumables

Vendor	Item	Part Number
For Nuclei Counting		
Nexcelom Biosciences	***ViaStain PI Staining Solution	CS1-0109-5mL
	***ViaStain AOPI Staining Solution	CS2-0106-5mL
	*Cellaca MX High-throughput Automated Cell Counter	MX-112-0127
	*Cellometer K2 Fluorescent Cell Counter	CMT-K2-MX-150
	PD100 Counting Chambers 1 case	CHT4-PD100-003
Biotium	***NucSpot 470	40083
	<i>Dilute the stock to 1:100 and mix 1:1 with the sample. For example, add 10 μl diluted dye to 10 μl sample.</i>	
Thermo Fisher Scientific	*Countess II FL Automated Cell Counter <i>Discontinued</i>	AMAQAF1000
	Countess Automated Cell Counting Chamber Slides	C10228
	*Countess 3 FL Automated Cell Counter	AMQAF2000
	Trypan Blue Stain (0.4%)	T10282
	***DAPI solution, 1 mg/mL	62248
***Choose either AOPI, NucSpot, PI, or DAPI solution. If the sample has no debris, Trypan Blue can be used.		
*Choose Countess II/3, Cellaca, Cellometer, or equivalent fluorescent counter.		

Illustrative Overview

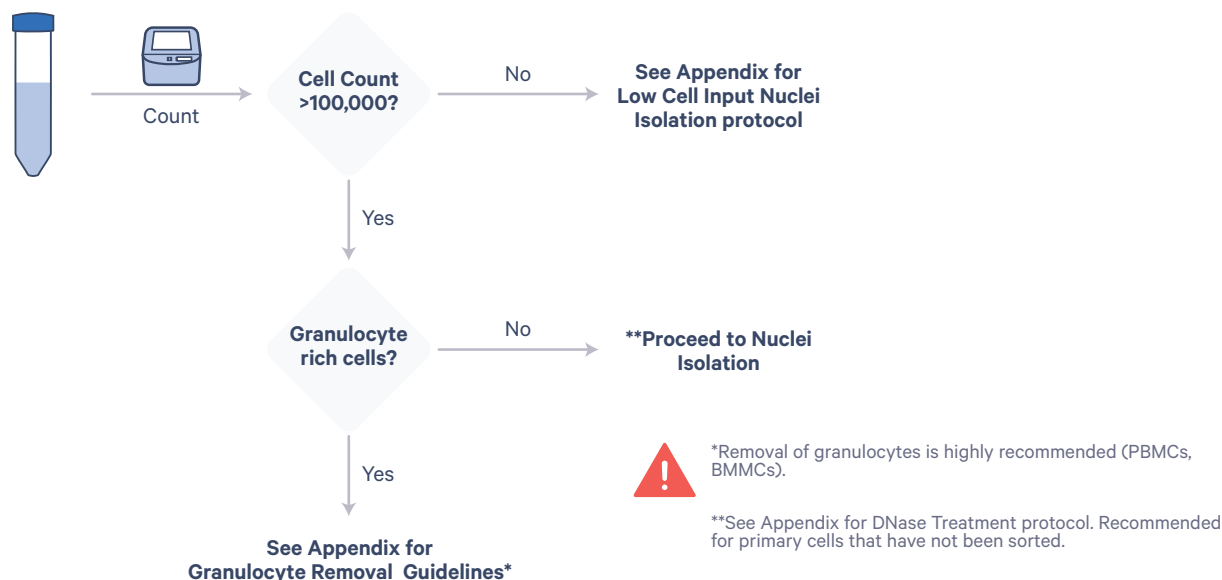
Frozen Cells

Thaw cells using the protocol for thawing cell lines or primary/fragile cells (step 1)

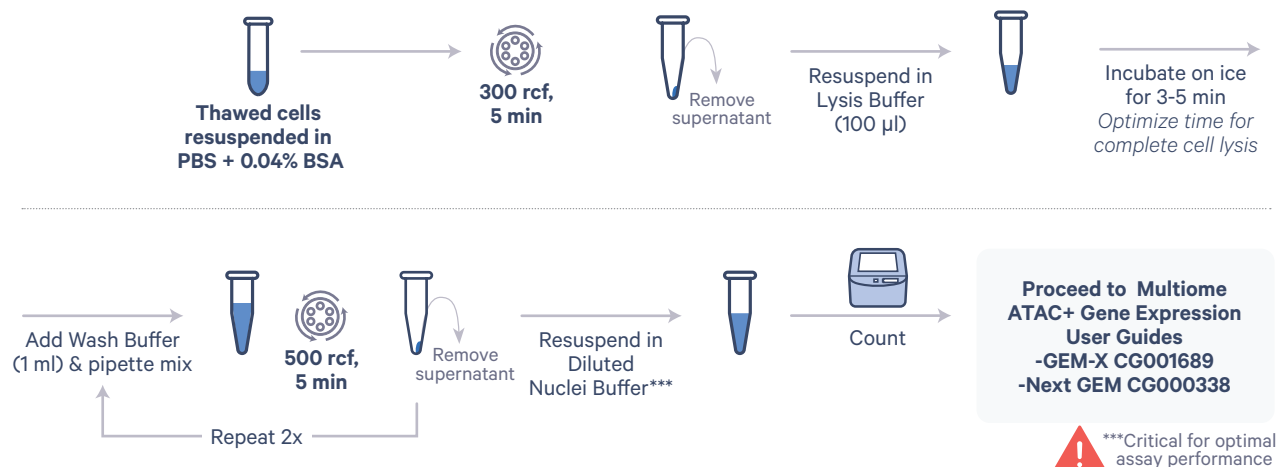
Fresh Cells

Wash cells with PBS + 0.04% BSA, determine cell count, and proceed to Nuclei Isolation (step 2)

1. Thaw Cells



2. Nuclei Isolation



Protocol

If using fresh cells, perform 1-2 washes with PBS + 0.04% BSA, determine cell count, and proceed to Nuclei Isolation (step 2a). Nuclei may be isolated from 100,000-1,000,000 cells using this protocol.

1. Thaw Cells (if using frozen cells)

Protocol for both primary cells/fragile cells (used for PBMCs) and cell lines (used for GM12878)

- Remove cryovials from storage, thaw in the water bath at **37°C** for **1-2 min**. Remove from the water bath when a tiny ice crystal remains.
- Using wide-bore pipette tip, add **1 ml** pre-warmed media (RPMI + 10% FBS) to the cryovial, pipette mix gently, and transfer to a 15-ml conical tube containing **9 ml** pre-warmed media (RPMI + 10% FBS).
- Centrifuge at **300 rcf** for **5 min** at **4°C**.
- Remove the supernatant without disrupting the cell pellet and resuspend in pre-chilled **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** at **4°C**.
- Remove the supernatant without disrupting the cell pellet and resuspend in **1 ml** PBS + 0.04% BSA.
- OPTIONAL Pass cell suspension through a 40 µm Flowmi Cell Strainer.
- Determine the cell concentration (see Appendix).
- 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).



Granulocyte removal before nuclei isolation is highly recommended for granulocyte-rich samples, such as PBMCs and BMMCs (see Appendix). Removing RNase-rich granulocytes prevents degradation of mRNA and excludes highly transposed DNA from Neutrophil Extracellular traps (NETs), resulting in cleaner data.

2. Nuclei Isolation

- Add 100,000-1,000,000 cells to a 1.5-ml microcentrifuge tube. Centrifuge at **300 rcf** for **5 min** at **4°C**.
- Remove the supernatant without disturbing the pellet. DO NOT leave behind more than 30 µl supernatant.
- 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 Automated Cell Counter/microscopy (see Results).

- Slowly add **1 ml** chilled Wash Buffer to the lysed cells against the tube wall. Pipette mix **5x**, without introducing bubbles.
- Centrifuge at **500 rcf** for **5 min** at **4°C**.
- Using a pipette, carefully remove the supernatant without disturbing the nuclei pellet. Leave behind no more than 30 µl supernatant.
- Repeat steps e-g two more times for a total of 3 washes.
- Based on cell concentration step 2a and assuming ~50% nuclei loss during cell lysis, resuspend in chilled Diluted Nuclei Buffer. Maintain on ice.

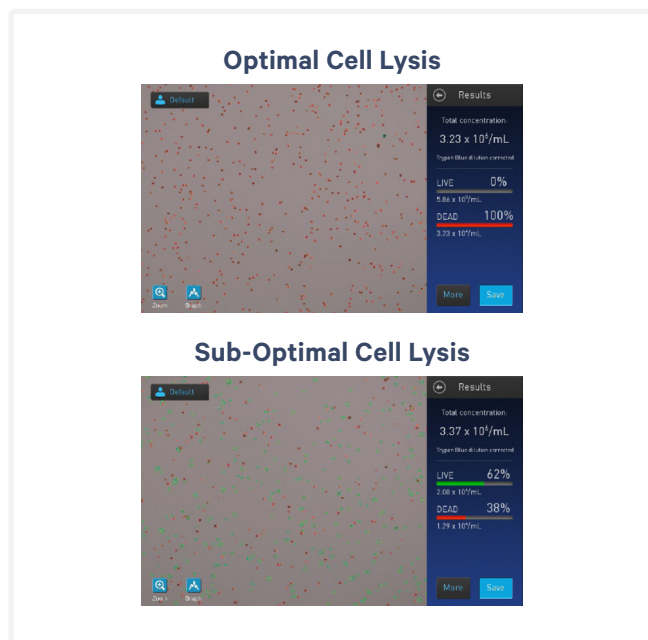


The resuspension in Diluted Nuclei Buffer is critical for optimal Single Cell ATAC assay performance. The composition of the Tris-based Diluted Nuclei Buffer, including Magnesium concentration, has been optimized for the Transposition and Barcoding steps in the Single Cell Multiome ATAC + GEX protocol.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

- 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.

- k. Determine the nuclei concentration (see Appendix).
- l. Proceed immediately to GEM-X Epi Multiome (CG001689) or Next GEM Multiome (CG000338) ATAC + Gene Expression User Guide.

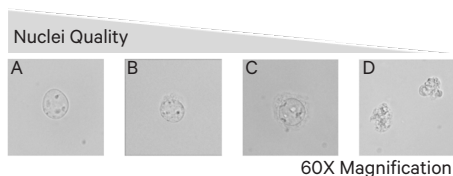
Results



Troubleshooting

Problem	Possible Solution
High fraction of non-viable cells in input material prior to starting nuclei isolation	<p>Optimize cell thawing to enhance sample quality</p> <p>Reduce fraction of dead cells. Refer to Demonstrated Protocol Removal of Dead Cells from Single Cell Suspensions for Single Cell RNA Sequencing (Document CG000093) or</p> <p>Use EasySep™ Dead Cell Removal Kit following manufacturer's guidance.</p> <p>Sort cells using flow cytometry</p> <p>Gently handle cell suspensions by following best practices and reduce cell processing times</p>
High fraction of viable cells post cell lysis	Incrementally increase the lysis time and monitor lysis efficacy microscopically
Difficult to count nuclei/excess debris	Use a fluorescent dye (ethidium-homodimer-1) and fluorescence compatible cell counter or microscope
Excessive debris	Sample may be cleaned by extra washes/filtering/density centrifugation/FACS (7-AAD stain)
Low nuclei recovery	Use a swing-bucket rotor for centrifugation steps

Nuclei Quality - Representative Images (Panel A: recommended quality)



Appendix

Nuclei Stock Concentration

Consult the relevant user guides for nuclei concentration guidelines:

GEM-X Epi Multiome ATAC + Gene Expression User Guide (CG001689)

or

Next GEM Multiome ATAC + Gene Expression User Guide (CG000338)

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.

- 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.
- Add 2,000–40,000 cells to a 0.2-ml tube in a total volume of **50 µl** PBS + 0.04% BSA.
- Centrifuge at **300 rcf** for **5 min** at **4°C**.
- Remove **45 µl** supernatant without touching the bottom of the tube to avoid dislodging cell pellet.
- Add **45 µl** chilled Lysis Buffer. Gently pipette mix **3x**.
- Incubate for **3-5 min*** on ice.

*Cryopreserved PBMCs were incubated for 3 min

*Cryopreserved cell lines were incubated for 5 min



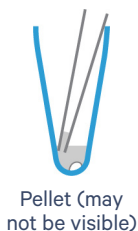
Optimize incubation time based on cell type. Sub-optimal or prolonged lysis times can both alter assay performance. Assess lysis efficacy via Automated Cell Counter/microscopy. See Results for optimal cell lysis.

- Add **50 µl** chilled Wash Buffer to the tube. DO NOT mix.
- Centrifuge at **500 rcf** for **5 min** at **4°C**.
- Remove **95 µl** supernatant without disrupting the nuclei pellet.
- Add **45 µl** chilled Diluted Nuclei Buffer to the pellet. DO NOT mix.
- 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- μ l pipette (set to 40 μ l), followed by removal with a 10- μ l pipette (set to 10 μ l).



- m. Resuspend the nuclei pellet in chilled Diluted Nuclei Buffer (pellet may not be visible).
 - Resuspend in **17 μ l** if proceeding to GEM-X Epi Multiome (CG001689)
 - Resuspend in **7 μ l** if proceeding to Next GEM Epi Multiome (CG000338)



The use of the Diluted Nuclei Buffer for nuclei suspension is critical for optimal 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 Multiome ATAC + GEX protocol. Suspension of nuclei in a different buffer may not be compatible with these protocol steps.

- n. Use **2 μ l** nuclei suspension mixed with **8 μ l** Diluted Nuclei Buffer and **10 μ l** staining dye to determine nuclei concentration
- o. Proceed immediately to GEM-X Epi Multiome (CG001689) or Next GEM Multiome (CG000338) ATAC + Gene Expression (GEX) User Guide.

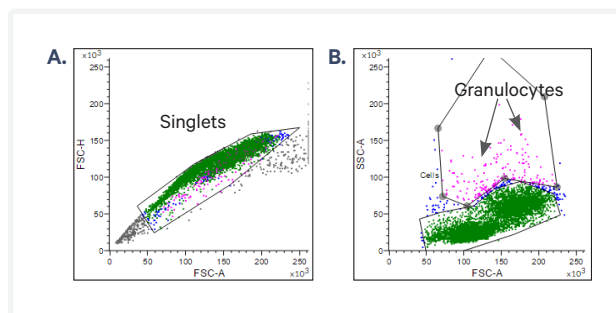
Granulocyte Removal Guidelines

Removal of granulocytes is highly recommended before nuclei isolation from granulocyte-rich samples, such as PBMCs and BMMCs.

Cell Sorting

- Cell sorting is not recommended if cell count is <100,000.
- After thawing and counting cells (step 1i), cells can be sorted using a 100 μ m nozzle (BD FACSMelody or comparable). No stain is needed for cell sorting. Granulocytes can be identified using side scatter.
- The first collection of sorted cells is for singlets as shown in the representative plot A.

- The second collection of sorted cells separates lymphocytes and monocytes (green cells) from granulocytes (pink cells) as shown in the representative plot B.



- The lymphocytes and monocytes fraction should be collected and used for isolating nuclei.
- Collect the sorted cells in a 5-ml FACS tube containing 500 μ l PBS + 0.04% BSA.
- Centrifuge the collected cells at 300 rcf for 5 min at 4°C.
- Remove the supernatant without disrupting the cell pellet and resuspend in 500 μ l PBS + 0.04% BSA.
- Determine the cell concentration (see Appendix).
- Proceed directly to Nuclei Isolation (step 2).

Bead-based Granulocyte Removal

- Use EasySep™ Direct Human PBMC Isolation Kit (EasySep Platform) to remove extra granulocytes in the sample before nuclei isolation. Based on estimated granulocyte number in the sample, adjust the reagent usage volume following manufacturer's guidance. A follow up check with FACS to ensure efficient granulocyte removal is highly recommended.

DNase Treatment

Recommended for primary cells prior to nuclei isolation. DNase treatment is not necessary if cells are being sorted prior to nuclei isolation.

Specific Reagents

DNase I, RNase-free (includes 10x Reaction Buffer with MgCl_2) from ThermoFisher Scientific, Part Number-EN0521

Preparation – Buffers

10X TBS	Stock	Final	5 ml
Tris-HCl (pH 7.4)	1 M	200 mM	1 ml
NaCl	5 M	1.5 M	1.5 ml
Nuclease-free Water	-	-	2.5 ml
DNase Solution	Stock	Final	1 ml
<i>Prepare fresh, maintain at 4°C</i>			
TBS	10X	1X	100 μl
10X Reaction Buffer with MgCl_2	10X	1X	100 μl
DNase I	1 U/ μl	0.1 U/ μl	100 μl
Nuclease-free Water	-	-	700 μl

Primary cells/fragile cells may have high amounts of ambient/background DNA. Treating the cells with DNase I prior to nuclei isolation can reduce the ambient DNA, which may improve the quality of libraries.

- a. Centrifuge the cells in a 2-ml microcentrifuge tube at **300 rcf** for **10 min** at **4°C**.



Using a 2-ml microcentrifuge tube and centrifuging for a longer time (10 min) is critical in maintaining an equal proportion of all cell types.

- b. Remove supernatant without disrupting the pellet and resuspend the pellet in **300 μl** DNase Solution.
- c. Pipette mix **5x** and incubate on ice for **5 min**.
- d. Add **1 ml** PBS + 0.04% BSA.
- e. Centrifuge cells at **300 rcf** for **10 min** at **4°C**.
- f. Remove supernatant without disrupting the pellet and resuspend the pellet in **1 ml** PBS + 0.04% BSA.
- g. Repeat steps d-e for a total of 2 washes.

- h. Pass cell suspension through a 40 μm Flowmi Cell Strainer.
- i. Determine the nuclei concentration (see Appendix).
- j. Proceed directly to Nuclei Isolation (step 2).

Nuclei Counting



Where recommended, accurate counting is essential for workflow execution. A fluorescent dye is strongly recommended. Trypan blue may be used in debris free samples only if fluorescent dye and counter are not available.

- Accurate sample counting is critical for achieving desired nuclei recovery. Table below shows the combination of counters and dyes tested for counting nuclei.
- It is strongly recommended that the sample be stained with a fluorescent dye such as PI staining solution and counted using an automated fluorescent cell counter or hemocytometer.
- The use of fluorescent dye during counting enables accurate quantification even in the presence of debris.
- Automated fluorescent cell counters are strongly recommended when counting nuclei.
- Ensure that the counter laser/filter setup is compatible with the fluorescent dye used.
- Ensure nuclei are well-focused under brightfield before switching to the fluorescent channel for counting.
- Increase exposure time to help adjust signal to noise during counting.
- Perform visual inspection to confirm that the counting number is accurate. For example, after obtaining the counting number, switch between

the brightfield and fluorescent channels to make sure the counts include minimal debris and the most nuclei.

Counting using PI Staining Solution

This protocol provides instructions for counting samples using PI staining solution and the Cellaca Counter to enable accurate quantification even in the presence of subcellular debris. The optimal cell/nuclei concentration for the Cellaca Counter is 100-10,000 cells/ μ l. Refer to manufacturer's instructions for details on operations.

- Add **25 μ l** PI Staining Solution into Mixing Row of Cellaca plate
- Gently mix the sample. If the sample is too concentrated, a 1:1 dilution in PBS can also be prepared. For example, add 15 μ l nuclei suspension to 15 μ l PBS. Ensure that this dilution factor is accounted for during counting. For example, because a 1:1 dilution was performed, the final cell concentration should be multiplied by two.
- Add **25 μ l** sample to Mixing Row of plate containing PI Staining Solution. Gently pipette mix 8x.
- Transfer stained sample to Loading Row of Cellaca plate.

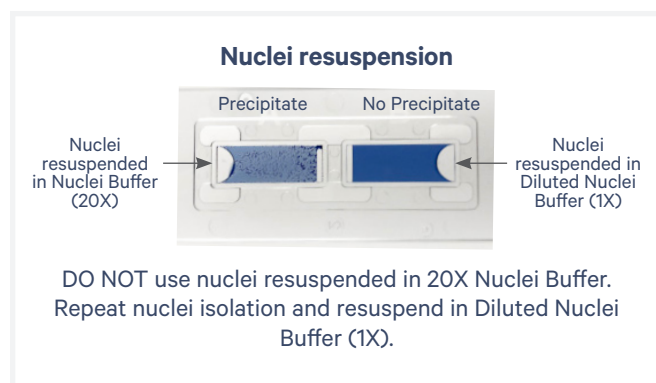
Samples stained with PI staining solution can also be counted using the Countess II/3 or K2 Automated Cell Counter. Refer to manufacturer's instructions for details.

Counter Type	Fluorescent Dye	Counting Comparison
Cellaca Range: 1×10^5 – 1×10^7 cells/ml Automated exclusion of debris from cell count	<ul style="list-style-type: none"> • Propidium Iodide • NucSpot 470 • DAPI 	Comparable counting results at both counting steps for all three dyes
Countess II FL/Countess 3 FL Range: 1×10^4 – 1×10^7 cells/ml (optimal 1×10^5 – 4×10^6) Manual debris exclusion from cell count post-image capture, using gates on the instrument program	<ul style="list-style-type: none"> • Propidium Iodide • NucSpot 470 • DAPI 	Comparable counting results at both counting steps for the three dyes
Cellometer K2 Range: 1×10^5 – 1×10^7 cells/ml Debris exclusion from cell count by adjusting instrument program settings before image capture	<ul style="list-style-type: none"> • Propidium Iodide • NucSpot 470 	Comparable counting results at both counting steps for the two dyes Propidium Iodide stained nuclei require longer exposure compared to NucSpot 470 but can still be relatively dimmer

Counting using Trypan Blue (Only for Debris-free Samples)

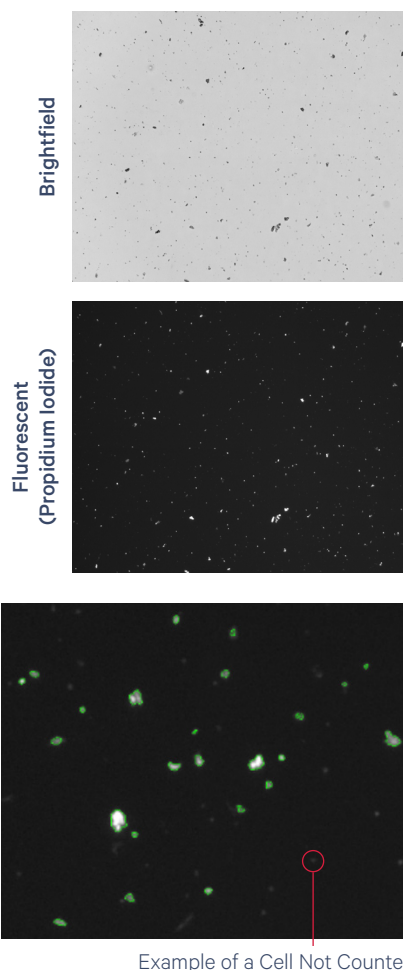
Debris-free samples (cells or nuclei suspensions) can also be counted using trypan blue. This protocol provides instructions for counting sample using trypan blue and a hemocytometer or Countess II Automated Cell Counter.

When counting nuclei, resuspend in diluted Nuclei Buffer (1X) and not in 20X nuclei buffer to prevent precipitation. See the example below.



- Mix **1 part** 0.4% trypan blue and **1 part** sample.
- Transfer **10 μ l** sample to a Countess II Cell Counting Slide chamber or a hemocytometer.
- Insert the slide into the Countess II Cell Counter and determine the cell concentration. Or if using a hemocytometer, count by placing the hemocytometer under the microscope.
- The majority of nuclei suspensions will be stained with trypan blue stain and appear nonviable.

Brightfield & Fluorescent Images from Automated Cell Counter - Samples Stained with Propidium Iodide



Brightfield image from Automated Cell Counter – Samples Stained with Trypan Blue

Fresh PBMCs



References

1. GEM-X Epi Multiome ATAC + Gene Expression User Guide (CG001689)
2. Chromium Next GEM Single Cell Multiome ATAC + Gene Expression User Guide (CG000338)

Document Revision Summary

Document Number	CG000365
Title	Nuclei Isolation for Single Cell Multiome ATAC + Gene Expression Sequencing
Revision	Rev E to Rev F
Revision Date	April 2026
Description of Changes	<p>Rev E and F updates:</p> <ul style="list-style-type: none">• Updated reagents table on page 2• Updated guidance for steps 1b, 1c & 1e on page 5• Removed section for primary cells/fragile cells• Updated guidance for steps 2b and 2e on page 5• Added granulocyte removal information on pages 2, 3 & 8-9• Added nuclei counting information on pages 3, 10-11• Updated References• Updated for general minor consistency of format, language, and terms throughout

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