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Low Cell Input Nuclei Isolation for Single Cell ATAC-Seq

Forked from [Nuclei Isolation for Single Cell ATAC-Seq](#)

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Disclaimer

Handling, use, storage, and disposal of human tissues must be conducted in a respectful manner in line with Human Tissue Authority (HTA) guidelines, reflecting the sensitive nature and origin of the material.

Ensure prior to start that collection, carriage, and receipt of tissues is compliant with all HTA guidelines, including Research Ethics Service/Committee approval, and thorough labelling and tracking of all human materials.

Abstract

This protocol describes the methodology of nuclei isolation for Single Cell ATAC Sequencing adopted from 10X Genomics guide CG000169 Revision D.

Materials

MATERIALS

- ☒ BSA Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7906
- ☒ FBS Invitrogen - Thermo Fisher
- ☒ Flowmi™ Cell Strainer 40 µm Bel-Art Catalog #H13680-0040
- ☒ PBS
- ☒ Tween-20
- ☒ RPMI 1640 Medium Thermo Fisher Scientific Catalog #11875093
- ☒ Single Cell ATAC Library and Gel Bead Kit 10x Genomics Catalog #PN-1000175
- ☒ Digitonin (5%) Thermo Fisher Scientific Catalog #BN2006
- ☒ Trizma® hydrochloride solution Merck MilliporeSigma (Sigma-Aldrich) Catalog #T2194-1L
- ☒ Nonidet™ P 40 Substitute Merck MilliporeSigma (Sigma-Aldrich) Catalog #74385
- ☒ Sodium chloride solution Merck MilliporeSigma (Sigma-Aldrich) Catalog #59222C
- ☒ MACS BSA Stock Solution Miltenyi Biotec Catalog #130-091-376
- ☒ Magnesium chloride solution Merck MilliporeSigma (Sigma-Aldrich) Catalog #M1028

Troubleshooting

Prepare diluted nuclei buffer

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Keep nuclei buffer on ice!

	Product	Stock	Final	Volume (1 ml)
	Nuclei buffer (20x) PN-2000153/2000207	20X	1x	50 ul
	Nuclease-free water			950 ul

**location at:

Prepare buffer A

2 Keep **buffer A** on ice and prepare fresh!

	Product	Stock	Final	Volume (4.9 4 ml)
	Tris-HCl (pH 7.4)	1M	10 mM	50 ul
	NaCl	5M	10 mM	10 ul
	MgCl ₂	1M	3 mM	15 ul
	BSA	10%	1%	500 ul
	Tween-20	10%	0.1%	50 ul
	Nuclease-free water			4.31 5 ml

Prepare wash buffer

3 Keep **wash buffer** on ice and prepare fresh!

	Product	Stock	Final	Volume (2 ml)
	Buffer A			1.97 6 ml
	Nuclease-free water			24 ul

**incubate Digotonin at 65 degree celcius to dissolve precipitate before use.

Prepare lysis buffer

- 4 Keep **lysis buffer** on ice and prepare fresh!

	Product	Stock	Final	Volume (2 ml)
	Buffer A			1.97 6 ml
	Nonidet P40 Substitute (if using Sigma (74385) 100% solution, prepare 10% stock	10%	0.1%	20 ul
	Digitonin	5%	0.01 %	4 ul

Additional buffers

- 5 PBS + 0.04% BSA (maintain at  4 °C)

	Product	Stock	Volume
	PBS		5 ml
	BSA (0.04%)	10%	20 ul

Nuclei isolation from fresh cells

- 6 Nuclei may be isolated from **2000-100,000 cells** using this protocol. Cells are already resuspended in PBS + 0.04% BSA.

Centrifuge cell suspension at **300 rcf** for 5min at  4 °C and re-suspend the cell pellet in 50 ul of PBS 0.04% BSA. Transfer 50 ul cell suspension to a 0.2ml tube. Proceed to next step.

- 7 Centrifuge at **300 rcf** for  00:05:00 at  4 °C .
- 8 Remove 45ul supernatant without touching the bottom of the tube to avoid dislodging the cell pellet.
- 9 Add  45 µL **chilled** Lysis Buffer. Gently pipette mix 3x.
- 10 Incubate for **4 min*** on **ice**.
*Time between 3-5min, depending on cell type.
- 11 Add  50 µL chilled Wash Buffer to the tube. DO NOT MIX!
- 12 Centrifuge at **500 rcf** for  00:05:00 at  4 °C .
- 13 Remove 95ul supernatant without disrupting the nuclei pellet.
- 14 Add  45 µL chilled Diluted Nuclei Buffer to the pellet. DO NOT MIX.
- 15 Centrifuge at **500 rcf** for  00:05:00 at  4 °C .
- 16 Remove the supernatant in 2 step without touching the bottom of the tube to avoid dislodging the nuclei pellet.
- 17 Resuspend the nuclei pellet in  5.5 µL chilled diluted nuclei buffer (pellet may not be visible).

18 **If original cell count <20,000 cells, do not need to count nuclei. Load everything into 10X.

Use $\text{2.5 } \mu\text{L}$ nuclei suspension mix with $\text{2.5 } \mu\text{L}$ Diluted Nuclei Buffer and $\text{5 } \mu\text{L}$ Trypan blue to determine cell count.

nuclei count= _____ $\times 2 \times 2 \times 10$ nuclei/ μL

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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

Example Calculation

Cell count at step 2a: **200,000**

Estimated nuclei count at step 2h (~50% loss): **100,000**

If targeting 5,000 Nuclei Recovery, nuclei pellet at step 2h may be resuspended in 30 μ L Diluted Nuclei Buffer for Nuclei Stock Concentration of 1,540-3,850 nuclei/ μ L (see Table above)

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Calculate volume of Nuclei Stock and Diluted Nuclei Buffer for a total volume of 5 µl

Volume of Nuclei Stock (μl) = $\frac{\text{Targeted Nuclei Recovery} \times 1.53 \text{ (Recovery efficiency factor)}}{\text{Nuclei Stock Concentration (nuclei/ } \mu\text{l})}$

Volume of Diluted Nuclei Buffer* (μl) = 5 μl - volume of Nuclei Stock (μl)

*Use ONLY Diluted Nuclei Buffer (Dilute Nuclei Buffer (PN-2000153) 1:20 in nuclease-free water)

Example Calculation

Targeted Nuclei Recovery = 4000 nuclei

Nuclei Stock Concentration = 2500 nuclei/ μl

Recovery efficiency factor 1.53

Volume of Nuclei Stock (μl) =

$\frac{\text{Targeted Nuclei Recovery} \times 1.53 \text{ (Recovery efficiency factor)}}{\text{Nuclei Stock Concentration (nuclei/ } \mu\text{l})} = \frac{4000 \times 1.53}{2500} = 2.45 \mu\text{l}$

Volume of Diluted Nuclei Buffer = 5 μl - 2.45 μl = 2.55 μl

Add calculated volumes of Diluted Nuclei Buffer and Nuclei Stock to the Transposition Mix in
step 1.1

21 Proceed to load about  5 μL of nuclei suspension for transposition step.

Prepare transposition mix

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- a. Prepare Transposition Mix on ice. Pipette mix 10x and centrifuge briefly.

Transposition Mix <i>Add reagents in the order listed</i>	PN	1X (μ l)	4X + 10% (μ l)	8X + 10% (μ l)
● ATAC Buffer	2000122	7.0	30.8	61.6
● ATAC Enzyme	2000123/ 2000138	3.0	13.2	26.4
Total	-	10.0	44.0	88.0

- b. Add 10 μ l Transposition Mix to a tube of a PCR 8-tube strip for each sample. Centrifuge briefly and maintain on ice.
- c. Refer to [Nuclei Concentration Guidelines](#) to calculate the volume of Nuclei Stock and Diluted Nuclei Buffer for a total volume of 5 μ l.
- d. Add the calculated volume of Diluted Nuclei Buffer to the Transposition Mix. Pipette mix. Centrifuge briefly.
- e. Gently pipette mix the Nuclei Stock. Add the calculated volume of the Nuclei Stock to the tube containing the Transposition Mix. Gently pipette mix 6x (pipette set to 10 μ l). DO NOT centrifuge.

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- a. Incubate in a thermal cycler using the following protocol.

Lid Temperature	Reaction Volume	Run Time
50°C	15 μ l	60 min
Step	Temperature	Time
Incubate	37°C	00:60:00
Hold	4°C	Hold

GEMS generation

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[CG000168_ChromiumSingleCell_ATAC_ReagentKits_UserGuide_RevC.pdf](#)