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Nuclei preparation from frozen tissue for Chromium Single Cell Multiome ATAC + Gene Expression (10x Genomics)

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Nathan Zemke¹, Bing Ren¹

¹University of California, San Diego



Adam Klie

UC San Diego

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Protocol status: Working

We use this protocol and it's working. Tissue collection for this protocol needs prior approval by the users' Institutional Review Board (IRB) or equivalent ethics committee

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Abstract

This protocol details nuclei preparation from frozen tissue for Chromium Single Cell Multiome ATAC + Gene Expression (10x Genomics). Tissue collection for this protocol needs prior approval by the users' Institutional Review Board (IRB) or equivalent ethics committee

Attachments



[841-276.pdf](#)

212KB

Materials

Reagents and consumables

Note

Prepare buffers fresh and leave  On ice .

NIM (Can be stored at  4 °C)

A	B	C	D
Reagent	Stock c	Final c	for 5 mL
Sucrose (S1888, Sigma)	1M	0.25M	1.25 mL
KCl (AM9640G, Invitrogen)	2M	25 mM	62.5 µl
MgCl ₂ (194698, Mp Biomedicals Inc)	1M	5 mM	25 µl
Tris-HCl, pH 7.5 (15567027, Thermo Fischer Scientific)	1M	10 mM	50 µl
Molecular biology water (46000-CM, Corning)	-	-	3.613 ml

NIM-DP (Make fresh)

A	B	C	D
Reagent	Stock c	Final c	For 2 mL
NIM buffer	1X	1X	1.9 ml
DTT (D9779, Sigma)	200 mM	1 mM	10 µl
Pierce Protease Inhibitor	50X	1X	40 µl
Recombinant RNAsin (Promega, PAN2515)	40 U/µl	1.0 U/µl	50 µl

NIM-DP-L

A	B	C	D
Reagent	Stock c	Final c	for 1 mL
NIM-DP	1X	1X	990 µl
Triton X-100 (Sigma, T8787-100ML)	10% (in water)	0.10%	10 µl

Sort buffer (SB) (Can be stored at 4C without RNasin or protease inh)

A	B	C	D
Reagent	Stock c	Final c	5 samples (500 uL each)
EDTA (Invitrogen, 15575020)	500 mM	1 mM	5 µl
Recombinant RNasin (Promega, PAN2515)	40 U/ul	1 U/ul	62.5 µl
Pierce Protease Inhibitor	50X	1X	50 µl
Fatty acid free BSA in PBS	10%	1%	250 uL
PBS	-	-	2.133 mL

Collection buffer (CB) (Can be stored at 4C without RNasin or protease inh)

A	B	C	D
Reagent	Stock c	Final c	5 samples (87.5 uL each)
Recombinant RNasin (Promega, PAN2515)	40 U/µl	5 U/µl	54.7 µl
Pierce Protease Inhibitor	50X	1X	8.75 µl
Fatty acid free BSA in PBS	10%	5%	218.75 µl
PBS	-	-	155.3 µl

5X OMNI (Permeabilization buffer) (Make fresh)

A	B	C	D
Reagent	Stock c	Final c	for 200 µL
Tris-HCl (pH 7.4) (15567027, Thermo Fischer Scientific)	1M	50 mM	10 µL
NaCl (Fischer, S271-3)	5M	50 mM	2 µl
MgCl ₂ (194698, Mp Biomedicals Inc)	1M	15 mM	3 µl
Tween-20 (Sigma, P7949-100ML)	10%	0.05%	1 µl
IGEPAL (Sigma, I8896)	10%	0.05%	1 µl
Digitonin (Promega, G9441)	2%	0.01%	0.5 µl
Fatty acid free BSA in PBS	10%	5%	100 µl



A	B	C	D
DTT (D9779, Sigma)	200mM	5mM	5 µl
Recombinant RNAsin (Promega, PAN2515)	40 U/µl	1U/µl	5 µl
Pierce Protease Inhibitor	50X	5X	20 µl
Molecular biology water (46000-CM, Corning)	-	-	52.5 µl

Wash Buffer (Make fresh)

A	B	C	D
Reagent	Stock c	Final c	For 2 mL
Tris-HCl (pH 7.4) (15567027, ThermoFischer Scientific)	1 M	10 mM	20 µL
NaCl (Fischer, S271-3)	5 M	10 mM	4 µl
MgCl ₂ (194698, Mp Biomedicals Inc)	1 M	3 mM	6 µl
Tween-20 (Sigma, P7949-100ML)	10%	0.10%	20 µl
Fatty acid free BSA in PBS	10%	1%	200 µl
DTT (D9779, Sigma)	200 mM	1 mM	10 µl
Recombinant RNAsin (Promega, PAN2515)	40 U/µl	1 U/µl	50 µl
Pierce Protease Inhibitor	50X	1X	40 µl
Molecular biology water (46000-CM, Corning)	-	-	1.65 ml

1X Nuclei Buffer (Make fresh)

A	B	C	D
Reagent	Stock c	Final c	100 uL
20X Nuclei Buffer (10X Genomics kit)	20X	1X	5 µl
DTT (D9779, Sigma)	200 mM	1 mM	0.5 µl
Recombinant RNAsin (Promega, PAN2515)	40 U/µl	1 U/µl	2.5 µl
Molecular biology water (46000-CM, Corning)	-	-	92 µl

- 7-AAD (Invitrogen, A1310)
- Sony Cell Sorter 100 µM Chip (Sony, LE-B3001)



- Sony Cell Sorter (Sony, SH800S)
- Eppendorf centrifuge (Eppendorf, 5920 R)
- 30 µm CellTrics (Sysmex, 04-0042-2316)
- 1 ml Dounce Tissue Grinder (Wheaton, 357538)
- 1.5 ml LoBind tubes (Eppendorf, 22431021)

☒ Sucrose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S1888**

☒ 2M KCl **Invitrogen - Thermo Fisher Catalog #AM9640G**

☒ Magnesium chloride, hexahydrate, cell culture reagent **MP Biomedicals Catalog #02194698-CF**

☒ 1M Tris-HCl pH 7.5 **Thermo Fisher Scientific Catalog #15567027**

☒ Molecular Biology Grade Water Tested to USP Sterile Purified Water Specifications **Corning Catalog #46-000-CM**

☒ DL-Dithiothreitol for molecular biology ≥98% (HPLC) ≥99% (titration) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D9779**

☒ Triton™ X-100 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #T8787-100ML**

☒ Invitrogen™ UltraPure™ 0.5M EDTA, pH 8.0 **Fisher Scientific Catalog #15-575-020**

☒ Sodium Chloride **Fisher Scientific Catalog #S271-3**

☒ TWEEN 20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P7949**

☒ Igepal **Merck MilliporeSigma (Sigma-Aldrich) Catalog #I8896**

☒ Digitonin **Promega Catalog #G9441**

☒ 7-AAD (7-Aminoactinomycin D) **Thermo Fisher Catalog #A1310**

☒ WHEATON® Dounce Tissue Grinder, 1 mL **DWK Life Sciences Catalog #357538**

☒ 1.5 mL LoBind tubes **Eppendorf Catalog #022431021**

Equipment

SH800S cell sorter	NAME
Sony	BRAND
SH800S	SKU
https://www.sonybiotechnology.com/us/instruments/sh800s-cell-sorter/specifications/	SPECIFICATIONS



Equipment

Centrifuge 5920 R	NAME
Eppendorf	BRAND
5920 R	SKU
https://www.eppendorf.com/us-en/eShop-Products/Centrifugation/Multipurpose-Centrifuges/Centrifuge-5920R-p-PF-240991	LIN K



Nuclei preparation

31m

- 1 Prechill any tubes, buffers or tools. Set the centrifuge to 4 °C .
- 2 For each sample to be homogenized, prepare a Dounce Tissue Grinder with 2 pestles ("Loose" and "Tight"). Remove from 70% ethanol storage, rinse with MilliQ water three times. Place mortars (buckets) in ice. Dry pestles with kimwipe and place on clean parafilm on top of ice to chill.
- 3 Remove excessive water collected at the bottom of mortars with p1000.

- 4 Add 990 μ L of NIM-DP and 10 μ L of Triton X-100 to each bucket to make 1 mL of NIM-DP-L. Pipette mix.



- 5 Tip tissue into mortar or resuspend tissue in 1 mL of NIM-DP-L buffer and transfer to a Dounce homogenizer.

Note

Be gentle and avoid introducing bubbles.

- 6 Homogenize the sample by using the Loose pestle (usually 5-10 strokes) followed by the tight pestle (usually 15-25). Switch pestles when most of the tissue has been broken up into small pieces and use the tight pestle to homogenize until the solution is uniform (no obvious particles).
- 7 Filter using a 30 μ m CellTrics filter into LoBind tube.
- 8 Centrifuge nuclei 1000 rcf, 4°C, 00:10:00 .
- 9 Discard the supernatant and gently resuspend pellet in 1 mL of NIM-DP. Centrifuge nuclei 1000 rcf, 4°C, 00:10:00 .
- 10 Add 7-AAD to Sort Buffer (1:1000) to a final concentration of 2 micromolar (μ M) .

10m



10m





11 Discard supernatant from pelleted nuclei. Gently resuspend pellet in  400 μL of sort buffer + 7-AAD by mixing with a p1000 pipette ~5 times or until no clumps are visible.



12 Sort 120,000 nuclei into a LoBind tube containing  87.5 μL of collection buffer.

13 Measure the total volume of the sorted nuclei in the collection buffer via reverse pipetting.

14 Add the appropriate amount of 5X permeabilization buffer for a final concentration of 1X, and gently pipette mix with p1000 5 times.

15 Incubate  On ice for  00:01:00 , then centrifuge  500 rcf, 4°C, 00:05:00 .

6m



16 Slowly remove the supernatant until 20-30 μL remains in the tube.



Note

Do not disturb pellet.

17 Slowly add  650 μL of Wash Buffer along wall of tube (try not to disturb pellet) and immediately centrifuge  500 rcf, 4°C, 00:05:00 .

5m



18 Prepare tubes for counting nuclei on hemocytometer by adding  7 μL of 1X Nuclei Buffer to each tube for a 1:16 dilution.



19 Very carefully remove the supernatant, switching to a p20 pipette once the supernatant volume is <  20 μL . Leave ~  1 μL to avoid disturbing the pellet.



20 Add  7 μL of 1X Nuclei Buffer and very gently resuspend pellet 4 times. Immediately take  1 μL and add to tube for counting containing  7 μL of 1X Nuclei Buffer.



21 Count nuclei by stain with  8 μL Trypan Blue (Invitrogen, T10282), count on a hemocytometer and record images from the microscope field. For calculating nuclei concentration of original stock multiply count average $\times 10 \times 16$ (dilution factor).



- 22 Refer to Chromium Single Cell Multiome ATAC + Gene Expression protocol (10x Genomics) Step 1. Load 18 - 20K nuclei per tagmentation.