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

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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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Keywords: chromium single cell multiome atac, nuclei preparation from frozen tissue, multiome atac, 10x genomic, chromium single cell, nuclei preparation, preparation from frozen tissue, tissue collection for this protocol, gene expression, frozen tissue, gene, tissue collection

Disclaimer

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

PDF

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)

	A	B	C	D
	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
	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

	A	B	C	D
	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	LINK

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 µL of NIM-DP and 10 µL 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 µm CellTrics filter into LoBind tube.

8 Centrifuge nuclei 1000 rcf, 4°C, 00:10:00. 10m

9 Discard the supernatant and gently resuspend pellet in 1 mL of NIM-DP. Centrifuge nuclei 1000 rcf, 4°C, 00:10:00. 10m

10 Add 7-AAD to Sort Buffer (1:1000) to a final concentration of [M] 2 micromolar (µM).

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 x 10 × 16 (dilution factor).

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