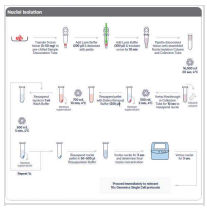

Aug 12, 2022

Chromium Nuclei Isolation for Single Cell Multiome ATAC + Gene Expression (CG000505)

10x Genomics[1]

[1]10x Genomics

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ABSTRACT

The Chromium Nuclei Isolation Kit is an all-in-one solution for the standardized isolation of nuclei from frozen tissue for use in 10x Genomics Single Cell assays. Frozen tissue samples are homogenized with a pestle in Lysis Buffer and passed through a column. Next, debris is removed via centrifugation in Debris Removal Buffer. The isolated nuclei are then washed and resuspended and loaded directly into compatible 10x Genomics Single Cell assays.

The Chromium Nuclei Isolation Kit streamlines the nuclei isolation process into a single workflow, allowing for increased efficiency, scalability through sample batching, and reduced experimental variability using 10x Genomics pre-formulated reagents. The protocol is designed to be compatible with a wide variety of tissue types and sizes.

This User Guide outlines the process for isolating Nuclei from frozen tissues for use in compatible 10x Genomics Single Cell assays. Refer to the Product Compatibility and Protocol Selector pages for additional information on choosing the appropriate nuclei isolation kit and protocol based on the intended downstream Single Cell assay.

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PROTOCOL CITATION

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KEYWORDS

scRNA-seq, scATAC-seq, 10x Genomics, nuclei, nuclei isolation

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GUIDELINES

Perform all steps at 4ºC

MATERIALS TEXT

1X Phospate-Buffered Saline without Ca2 and Mg2 **Corning Catalog #21-040-CV**

10% Bovine Serium Albumin **Merck Millipore Sigma Catalog #A1595**

RNase Inhibitor **10x Genomics Catalog #2000565**

20X Nuclei buffer **10x Genomics Catalog #2000207**

Reducing Agent B **10x Genomics Catalog #2000087**

Molecular Grade Nuclease-free Water **Thermo Fisher Scientific Catalog #AM9937**

Lysis Reagent **10x Genomics Catalog #2000558**

Surfactant A **10x Genomics Catalog #2000559**

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Pestle **10x**

Genomics Catalog #2000561

Nuclei Isolation column **10x**

Genomics Catalog #2000562

Collection Tube **10x**

Genomics Catalog #2000563

Sample Dissociation Tube **10x**

Genomics Catalog #2000564

DNA LoBind Tube 2.0

mL **Eppendorf Catalog #022431048**

15 mL centrifuge

tube **Corning Catalog #CLS430791**

50 mL centrifuge

tube **Corning Catalog #CLS430829**

VitaStain AOPI Staining

Solution **Nexcelom Catalog #CS2-0106-5ml**

Cellaca MX High-throughput Automated Cell Counter

cell counter

Nexcelom **MX0112-0127**

VWR ANALOG VORTEX MIXER

VWR **10153-838**

With tube insert

Centrifuge

Bench centrifuge

Eppendorf **5424**

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SAFETY WARNINGS

None

Buffer Preparation 10m

10m

1 Prepare Lysis Buffer (500 µL/rxn)

A	B	C	D	E
	PN	1X + 10% (µL)	4X + 10% (µL)	8X + 10% (µL)
Lysis Reagent	2000558	550	2200	4400
Reducing Agent B	2000087	0.55	2.2	4.4
Surfactant A	2000559	5.5	22	44
Total		556.05	2224.2	4448.4

2 Prepare Debris Removal Buffer (500 µL/rxn)

A	B	C	D	E
	PN	1X + 10% (µL)	4X + 10% (µL)	8X + 10% (µL)
Debris Removal Reagent	2000560	550	2200	4400
Reducing Agent B	2000087	0.55	2.2	4.4
Total		550.55	2202.2	4404.4

3 Prepare Wash Buffer (2 mL/rxn)

A	B	C	D	E
	PN	1X + 10% (µL)	4X + 10% (µL)	8X + 10% (µL)
1X PBS	-	1925	7700	15400
10% BSA	-	220	880	1760
RNase Inhibitor	2000565	55	220	440
Total		2200	8800	1760

4 Prepare Resuspension Buffer (1 mL/rxn)

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A	B	C	D	E
	PN	1X + 10% (µL)	4X + 10% (µL)	8X + 10% (µL)
20X Nuclei Buffer	2000207	55	220	440
Reducing Agent B	2000087	1.1	4.4	8.8
Nuclease-free Water	-	1016	4066	8131
RNase Inhibitor	2000565	27.5	110	220
Total		1099.6	4400.4	8799.8

Nuclei Isolation: Tissue Dissociation 10m

5 Pre-chill centrifuge to 4°C and place reagents and tubes on ice as indicated in the Get Started guide. Label tops and sides of tubes, as well as tops of spin columns, before starting protocol. Perform all protocol steps on ice and centrifugation steps at 4°C.

6 Prepare Single Cell Multiome ATAC + Gene Expression buffers according to Buffer Preparation section and place on ice.

7 Place Sample Dissociation Tube(s) on dry ice.

8 Obtain frozen tissue sample(s) and place immediately on dry ice.

9 Transfer frozen tissue (3–50 mg) to pre-chilled Sample Dissociation Tube.

10 Transfer Sample Dissociation Tubes(s) to wet ice. Add 200 µl Lysis Buffer to Sample Dissociation Tube. Dissociate tissue with plastic pestle until homogeneous. For multiple samples, add Lysis Buffer to each tissue and then proceed to dissociate one at a time. Perform tissue dissociation on ice. Use one pestle per sample. DO NOT discard pestles until nuclei isolation process is complete.

11 Add 300 µl Lysis Buffer. Pipette mix 10x. If pipette tip clogs with unhomogenized tissue, continue to dissociate tissue with the pestle until able to pipette mix.

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12 Incubate on ice for 10 min.

13 Pipette dissociated tissue into pre-chilled Nuclei Isolation Column assembled with Collection Tube using pipette set to 500 µl. Transfer all liquid from Dissociation Tube to Nuclei Isolation Column to avoid nuclei loss.

14 Centrifuge at 16,000 rcf for 20 sec at 4°C. See Tips & Best Practices on page 14 for centrifuge loading guidance.

15 Discard column. Flowthrough in the Collection Tube will contain nuclei. Vortex 10 sec at 3,200 rpm or max speed to resuspend nuclei. Flowthrough may appear opaque or cloudy. This is normal and it is safe to proceed.

16 Centrifuge Collection Tube for 3 min at 500 rcf at 4°C. Carefully discard supernatant using a pipette without disturbing nuclei pellet. Leave behind a small fraction (~200 µl) of supernatant if nuclei pellet is not apparent. Position tubes with hinges facing in same direction within the centrifuge, which ensures that the pellet is consistently in the same place (opposite the hinge) following centrifugation.

Nuclei Isolation: Isolation and cleanup 45m

17 Resuspend nuclei pellet in 500 µl Debris Removal Buffer. Gently pipette mix at least 15x, continuing until no pellet can be visualized.

18 Centrifuge at 700 rcf for 10 min at 4°C. Carefully discard supernatant using a pipette without disturbing nuclei pellet. Leave behind a small fraction (~200 µl) of supernatant if nuclei pellet is not apparent.

19 Resuspend nuclei pellet in 1 ml of Wash Buffer.

20 Centrifuge at 500 rcf for 5 min at 4°C. Carefully discard supernatant using a pipette without disturbing nuclei pellet. Leave behind a small fraction (~200 µl) of supernatant if nuclei pellet is not apparent.

21 Resuspend nuclei pellet in 1 ml of Wash Buffer.

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22 Centrifuge at 500 rcf for 5 min at 4°C. Carefully discard as much supernatant as possible using a pipette without disturbing nuclei pellet. Leave behind a small remaining volume if the pellet is not visible.

23 Resuspend nuclei pellet in 50–500 µl Resuspension Buffer, depending on expected recovery for input tissue type and mass. Refer to Nuclei Recovery section of Tips & Best Practices for information on typical nuclei recovery. Gently pipette mix 15x using an appropriate pipette for resuspension volume.

24 Vortex nuclei for 3 sec at 3,200 rpm or max speed immediately prior to counting to ensure accurate nuclei count. Pulse spin the tube after vortexing to collect liquid at bottom of tube. DO NOT pulse spin the tube for more than 1 second to ensure that nuclei do not pellet at the bottom of the tube.

25 Determine nuclei concentration using AOPI or Ethidium Homodimer-1 fluorescent staining dyes and dilute if necessary for target nuclei load. Follow recommendations for nuclei counting as outlined in the Tips & Best Practices on page 19 of this document. Adjust nuclei concentration as necessary for intended downstream assay.

26 Vortex nuclei for 3 sec at 3,200 rpm or max speed. Pulse spin the tube after vortexing to collect liquid at bottom of tube. DO NOT pulse spin the tube for more than 1 second to ensure that nuclei do not pellet at the bottom of the tube.

27 Keep samples on ice and proceed immediately to relevant 10x Genomics User Guide.

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