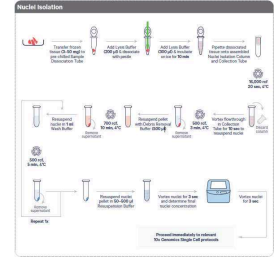


## 🌐 Chromium Nuclei Isolation for Single Cell Multiome ATAC + Gene Expression (CG000505)

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<sup>1</sup>10x Genomics

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**Protocol status:** Working

**We use this protocol and it's working**

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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.

## Guidelines

Perform all steps at 4°C

## Materials

- ☒ 1X Phosphate-Buffered Saline without Ca<sup>2</sup> and Mg<sup>2</sup> **Corning Catalog #21-040-CV**
- ☒ 10% Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich) 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**
- ☒ 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**

## Equipment

Cellaca MX High-throughput Automated Cell Counter	NAME
cell counter	TYPE
Nexcelom	BRAND
MX0112-0127	SKU

## Equipment

VWR ANALOG VORTEX MIXER

NAME

VWR

BRAND

10153-838

SKU

With tube insert

SPECIFICATIONS

## Equipment

Centrifuge

NAME

Bench centrifuge

TYPE

Eppendorf


BRAND

5424

SKU

## Troubleshooting

## Safety warnings

 None



## Buffer Preparation

10m

### 1 Prepare Lysis Buffer (500 $\mu$ L/rxn)

10m

	A	B	C	D	E
		PN	1X + 10% ( $\mu$ L)	4X + 10% ( $\mu$ L)	8X + 10% ( $\mu$ L)
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 $\mu$ L/rxn)

	A	B	C	D	E
		PN	1X + 10% ( $\mu$ L)	4X + 10% ( $\mu$ L)	8X + 10% ( $\mu$ L)
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% ( $\mu$ L)	4X + 10% ( $\mu$ L)	8X + 10% ( $\mu$ L)
1X PBS		-	1925	7700	15400
10% BSA		-	220	880	1760



	A	B	C	D	E
	RNase Inhibitor	2000565	55	220	440
	Total		2200	8800	1760

#### 4 Prepare Resuspension Buffer (1 mL/rxn)

	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  $\mu$ l 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  $\mu$ l Lysis Buffer. Pipette mix 10x. If pipette tip clogs with unhomogenized tissue, continue to dissociate tissue with the pestle until able to pipette mix.
- 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  $\mu$ l. 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  $\mu$ l) 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  $\mu$ l 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  $\mu$ l) 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.
- 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.