10x GENOMICS

Sample Prep User Guide | CG000505 | Rev A

Chromium Nuclei Isolation Kit

For use with:

Chromium Nuclei Isolation Kit
16 rxns, PN-1000493

Chromium Nuclei Isolation Kit with RNase Inhibitor
16 rxns, PN-1000494

Notices

Document Number

CG000505 | Rev A

Legal Notices

Instrument & Licensed Software Updates Warranties

Updates to existing Instruments and Licensed Software may be required to enable customers to use new or existing products. In the event of an Instrument failure resulting from an update, such failed Instrument will be replaced or repaired in accordance with the 10x Limited Warranty, Assurance Plan or service agreement, only if such Instrument is covered by any of the foregoing at the time of such failure. Instruments not covered under a current 10x Limited Warranty, Assurance Plan or service agreement will not be replaced or repaired.

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Document Revision Summary

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Title

Chromium Nuclei Isolation Reagent Kits Sample Prep User Guide

Revision

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May 2022

Specific Changes

General Changes

Table of Contents

Table of Contents

10x GENOMICS

Introduction

Objective

Protocol Steps & Timing

Product Compatibility

Additional Reagents, Kits, & Equipment

Recommended Pipette Tips

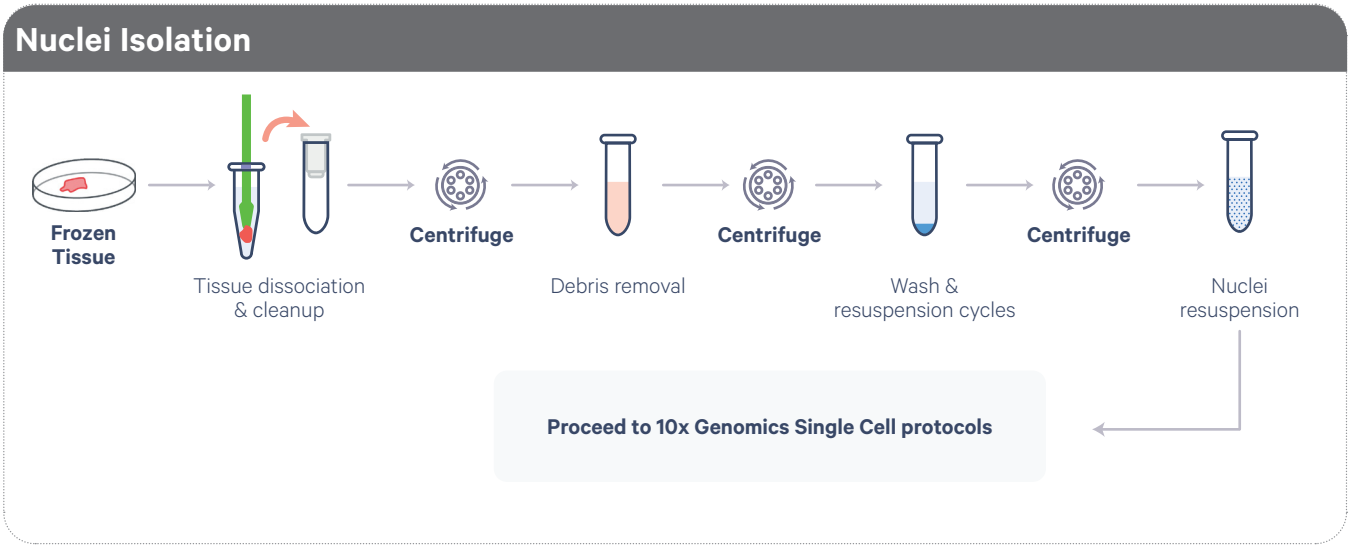
Objective

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.

High Level Overview of Nuclei Isolation Workflow



Protocol Steps & Timing

The table below provides an overview of the nuclei isolation workflow steps and timing. This protocol is to be executed without any stopping points.

Steps	Timing
Buffer Preparation	10 min
Nuclei Isolation Process	
Tissue Dissociation	10 min
Nuclei Isolation & Cleanup	45 min
Sample QC	10 min

*Keep samples on ice following QC. Proceed **immediately** to relevant 10x Genomics Single Cell protocol.*

Product Compatibility

The Chromium Nuclei Isolation Kit is compatible with 10x Genomics Single Cell assays. This table outlines the supported products for the Chromium Nuclei Isolation Kits, as well as the reagents and consumables included in each kit. Consult the Protocol Selector page of this document for additional information regarding kit and product selection.

Chromium Nuclei Isolation Kit	Kit Components	Supported Products
Chromium Nuclei Isolation Kit (PN-1000493)	• Chromium Nuclei Isolation Reagents, 16 rxns *(PN-1000447)* • Chromium Nuclei Isolation Consumables, 16 rxns *(PN-1000448)* • Reducing Agent B *(PN-1000450)*	• Single Cell ATAC
Chromium Nuclei Isolation Kit with RNase Inhibitor (PN-1000494)	• Chromium Nuclei Isolation Reagents, 16 rxns *(PN-1000447)* • Chromium Nuclei Isolation Consumables, 16 rxns *(PN-1000448)* • Reducing Agent B *(PN-1000450)* • RNase Inhibitor Kit *(PN-1000449)*	• Single Cell 3’ Gene Expression • Single Cell 5’ Gene Expression • Single Cell Multiome ATAC + Gene Expression • Chromium Fixed RNA Profiling

Additional Kits, Reagents & Equipment

The items in the table below have been validated by 10x Genomics and are highly recommended for the Nuclei Isolation protocol. Substituting materials may adversely affect system performance. This list does not include standard laboratory equipment, such as water baths, centrifuges, vortex mixers, pH meters, freezers, etc.

Item	Description	Supplier	Part Number
Plastics			
2-ml Tubes	DNA LoBind Tubes 2.0 ml	Eppendorf	022431048
15-ml Tubes	Corning 15 ml centrifuge tubes	Corning	CLS430791
50-ml Tubes	Corning 50 ml centrifuge tubes	Corning	CLS430829
Kits & Reagents			
10% BSA	Bovine Serum Albumin in DPBS (10%) *(alternatively, use MACS BSA Stock Solution)*	Millipore-Sigma *Miltenyi Biotec*	A1595 *130-091-376*
1X PBS	Phosphate-Buffered Saline without Calcium & Magnesium	Corning	21-040-CV
Nuclease-free Water	Molecular Grade Nuclease-free Water	Thermo Fisher Scientific	AM9937
Cell Counting			
Nucleic Acid Staining Fluorescent Dye	VitaStain AOPI Staining Solution *(alternatively, use Ethidium Homodimer-1)*	Nexcelom *Thermo Fisher Scientific*	CS2-0106-5ml *E1169*
Cell Counter	Cellaca MX High-throughput Automated Cell Counter *(alternatively, use any cell counter with fluorescent capabilities)*	Nexcelom	MX-112-0127
Equipment			
Vortex	Vortex Mixer	VWR	10153-838
Centrifuge	Refrigerated Eppendorf Centrifuge *(alternatively, use any equivalent centrifuge)*	Millipore-Sigma	5427R or 5424R

Recommended Pipette Tips

10x Genomics recommends using only validated emulsion-safe pipette tips for all Single Cell protocols, including the Chromium Nuclei Isolation protocol. Rainin pipette tips have been extensively validated by 10x Genomics and are highly recommended for all single cell assays. If Rainin tips are unavailable, any of the listed alternate pipette tips validated by 10x Genomics may be used.

Supplier	Description	Part Number (US)
Recommended Pipettes & Pipette tips		
Rainin (pipettes)	Pipet-Lite Multi Pipette L8-50XLS+	17013804
	Pipet-Lite Multi Pipette L8-200XLS+	17013805
	Pipet-Lite Multi Pipette L8-10XLS+	17013802
	Pipet-Lite Multi Pipette L8-20XLS+	17013803
	Pipet-Lite LTS Pipette L-2XLS+	17014393
	Pipet-Lite LTS Pipette L-10XLS+	17014388
	Pipet-Lite LTS Pipette L-20XLS+	17014392
	Pipet-Lite LTS Pipette L-100XLS+	17014384
	Pipet-Lite LTS Pipette L-200XLS+	17014391
	Pipet-Lite LTS Pipette L-1000XLS+	17014382
Rainin (pipette tips)	Tips LTS 200UL Filter RT-L200FLR	30389240
	Tips LTS 1ML Filter RT-L1000FLR	30389213
	Tips LTS 20UL Filter RT-L10FLR	30389226
Alternate Recommendations *(If Rainin pipette tips are unavailable, any of the listed pipette tips may be used)*		
Eppendorf (pipettes)	Eppendorf Research plus, 8-channel, epT.I.P.S. Box, 0.5 – 10 µL	3125000010
	Eppendorf Research plus, 8-channel, epT.I.P.S. Box, 10 – 100 µL	3125000036
	Eppendorf Research plus, 8-channel, epT.I.P.S. Box, 100 – 300 µL	3125000052
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 0.1 – 2.5 µL	3123000012
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 0.5 – 10 µL	3123000020
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 2 – 20 µL	3123000039
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 2 – 200 µL	3123000055
	Eppendorf Research plus, 1-channel, epT.I.P.S.® Box, 100 – 1000 µL	3123000063
Eppendorf (pipette tips) *Compatible with Eppendorf pipettes only*	ep Dualfilter T.I.P.S., 2-20 µL	0030078535
	ep Dualfilter T.I.P.S., 2-200 µL	0030078551
	ep Dualfilter T.I.P.S., 2-1,000 µL	0030078578
Labcon*	ZAP SLIK 20 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1143-965-008
	ZAP SLIK 200 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1144-965-008
	ZAP SLIK 1000 µL Low Retention Aerosol Filter Pipet Tips for Rainin LTS	4-1145-965-008
Biotix*	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 0.1-20uL	63300931
	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 200uL	63300001
	xTIP4 Racked Pipette Tips, Rainin LTS Pipette Compatible, 1000uL	63300003

**Compatible with Rainin pipettes*

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Tips & Best Practices

Icons

Tips & Best Practices section includes additional guidance

Signifies critical step requiring accurate execution

Troubleshooting section includes additional guidance

Plastics

- Use recommended plastic consumables when isolating nuclei as some plastics can introduce fibers into reagents, buffers, and solutions, leading to microfluidic failures.

General Reagent Handling

- Fully thaw and thoroughly mix reagents before use.
- Keep all reagents on ice during setup and use. Promptly move reagents back to the recommended storage after use.
- Note that an excess of 10% of 1 reaction value is calculated for all buffer reagent volumes in this User Guide.
- Thoroughly mix samples before each step.
- If provided Lysis Reagent and Debris Removal Buffers appear cloudy or contain precipitate, warm the tubes to **40°C** and swirl until the buffers become clear again.

Pipette Tips & Calibration

- Follow manufacturer's calibration and maintenance schedules.
- Use only recommended pipette tips.

Experimental Setup

- Minimize exposure of reagents, columns, and tubes to sources of particles and fibers, laboratory wipes, frequently opened flip-cap tubes, clothing that sheds fibers, and dusty surfaces.
- Execute steps without pause or delay.
- Pre-chill centrifuge to **4°C** before starting protocol. Ensure centrifuge is balanced prior to loading samples.
- If using a fixed angle rotor centrifuge, orient tube caps inward to avoid breaking of caps during centrifugation steps. Alternatively, use empty balance tubes to pin the collection tube cap as indicated in the image below.

Orient Tube Caps Facing Inward During Centrifugation

*Caps of tubes are angeled toward the balance tubes to prevent them from turning outward due to the centrifugal force.

- Label tops and sides of all tubes, as well as tops of spin columns, for clear identification of samples.
- If centrifuge model does not allow for **20 sec** spin during first centrifugation step of Nuclei Isolation Protocol, set it to **30 sec** and stop spin at **20 sec**.

Tissue Handling & Storage

- Work quickly and minimize handling during all tissue processing steps.
- Wash tissues in a clean glass petri dish with cold PBS upon harvest and absorb excess blood using a laboratory wipe.
- Cut tissues into small pieces (i.e. the size of a rice grain) for ease of freezing and place in a cryovial.
- To flash freeze, either submerge the cryovial in liquid nitrogen or a liquid-nitrogen cooled bath (e.g. isopentane) or place the tube deep in a bucket of dry ice. Wait at least 2–3 minutes for the tissue to freeze all the way through, and transfer the tube containing the tissue to vapor phase liquid nitrogen for long-term storage.
- Tissues should be stored **long-term** in a cryovial in **liquid nitrogen** for best results. Tissues can be stored **short-term** (1–2 days) at **-80°C** if needed.
- Once removed from liquid nitrogen, tissues should be maintained at **-80°C** or on **dry ice** until use.

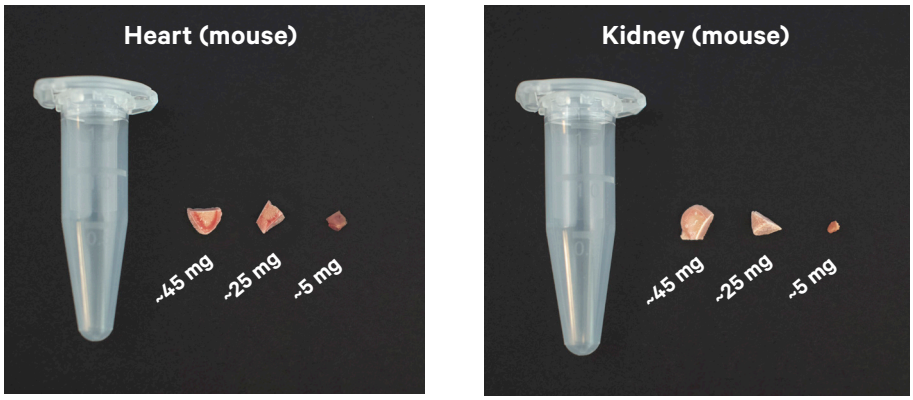
Thawing tissue prior to dissociation is NOT recommended as this can result in RNA degradation.

Tissue Dissociation

- Weigh input tissues before proceeding with tissue dissociation. The Chromium Nuclei Isolation Kit requires input tissue masses between **3–50 mg**.

TIPS *Start with sufficient material. Refer to Nuclei Concentration for Optimal Performance and Nuclei Recovery sections for guidance on nuclei recovery.*

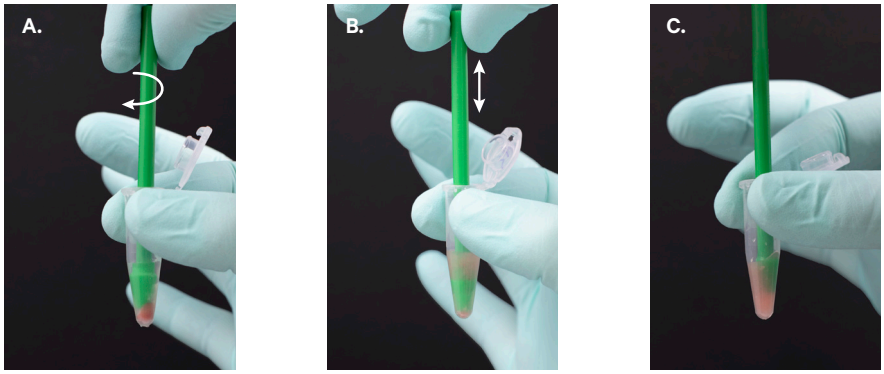
Example Tissues & Masses



- If tissue mass is **>50 mg**, cut tissue into smaller sections on a dry ice-cooled glass petri dish and place each section into a Sample Dissociation Tube. The resulting nuclei from each isolation can be combined at the point of resuspension immediately prior to counting and loading of the downstream Single Cell assay.
- For difficult to dissociate, fibrous tissues, pre-chopping tissue into smaller pieces **(≤10 mg)** will aid in dissociation and increase nuclei recovery.
- Dissociate the tissue in the Lysis Buffer by grasping the pestle between thumb and fingers. Twist the pestle continuously within the tube. Alternate between pushing the pestle down and lifting the pestle up to allow the tissue to slide between the wall of the tube and the pestle.
- Continue dissociating the tissue until the lysate appears homogeneous and no large pieces of tissue remain.
- Use only the pestles provided in this kit for tissue dissociation steps.
- If pestles are accidentally dropped or discarded, they may be washed with 70–80% ethanol before usage.

Tissue Dissociation
Contd.

Using the Pestle for Tissue Dissociation*



A. Grasp pestle between thumb and fingers. Twist the pestle continuously within the tube.

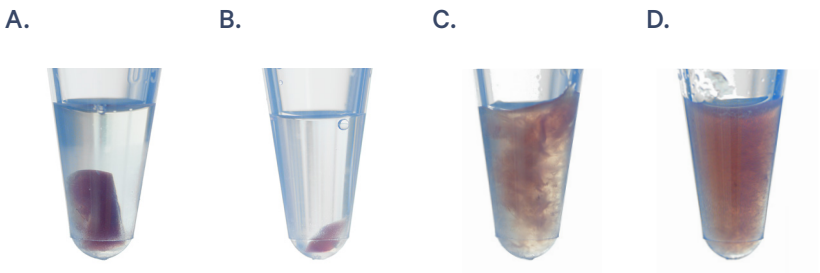
B. Alternate between pushing the pestle down and lifting the pestle up.

C. Allow tissue to slide down wall of tube.

**Repeat these steps until tissue lysate is homogeneous.*

- Note that for many sample types there may be a small amount of white or opaque particulate that remain in the tube following tissue dissociation. These debris may be left behind when transferring the lysate to the spin column in subsequent steps.

Stages of Tissue Dissociation



A. Large tissue, not dissociated

B. Small tissue, not dissociated

C. Partially dissociated

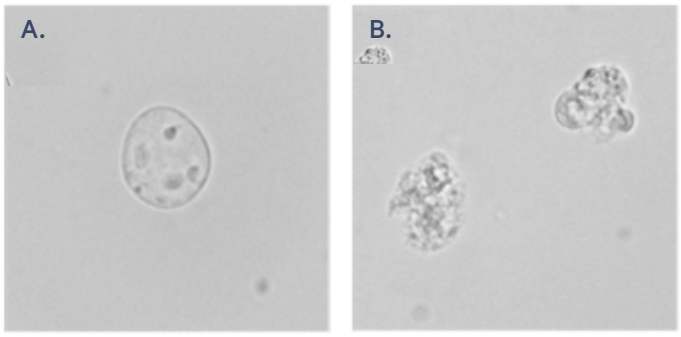
D. Fully dissociated, homogeneous tissue lysate

- Tissue dissociation is sample dependent. Soft tissues may only require a few pestle strokes for dissociation, while more fibrous tissue will require more pestle strokes to fully dissociate tissue. No large tissue chunks should remain after tissue dissociation and tissue homogenate should be able to easily pass through a P1000 pipette tip without clogging.

Lysis Conditions

- Nuclei lysis should be carried out on ice using chilled reagents and tools.
- Overlysis of nuclei occurs when samples are suspended in Lysis Buffer for an extended period of time. Avoid overlysis of nuclei which can lead to leakage of nuclear content and high levels of background signal.
- Nuclei with an intact membrane will appear round and smooth, while nuclei with a compromised membrane will appear "ruffled", an indication of blebbing.
- The Chromium Nuclei Isolation Kit lysis conditions have been shown to be compatible across a wide variety of tissue types. Follow the protocol as indicated and QC nuclei before attempting to perform additional lysis condition optimization.

Assessing Nuclei Quality



Good nuclei quality | Poor nuclei quality with blebbing

40x Magnification

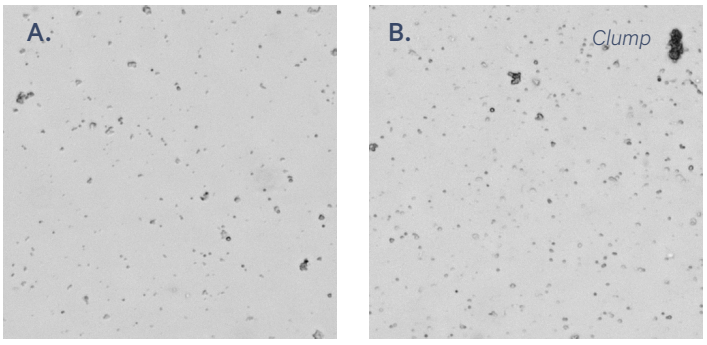
Debris & Aggregate Removal

- A high-quality nuclei suspension with minimal aggregates and debris is critical for single cell sequencing.
- For high debris tissues (e.g. brain), starting with smaller tissue input **(<25 mg)** will reduce levels of debris and background in final nuclei suspension.
- Aggregates can be caused by insufficient resuspension of nuclei between steps of this protocol. Vortex nuclei samples where indicated during resuspension and wash steps.
- Debris and clumping can also be a sign of overlysis. Lysis optimization may be needed if vortexing steps were followed and clumping is not resolved.

TIPS

Some level of small debris is acceptable during the nuclei isolation process and is not expected to negatively impact data quality.

Debris & Aggregates



Acceptable sizes of debris and aggregates

Unacceptable sizes of debris and aggregates

Analyzed on the Cellaca MX Cell Counter

Nuclei Visualization & Counting

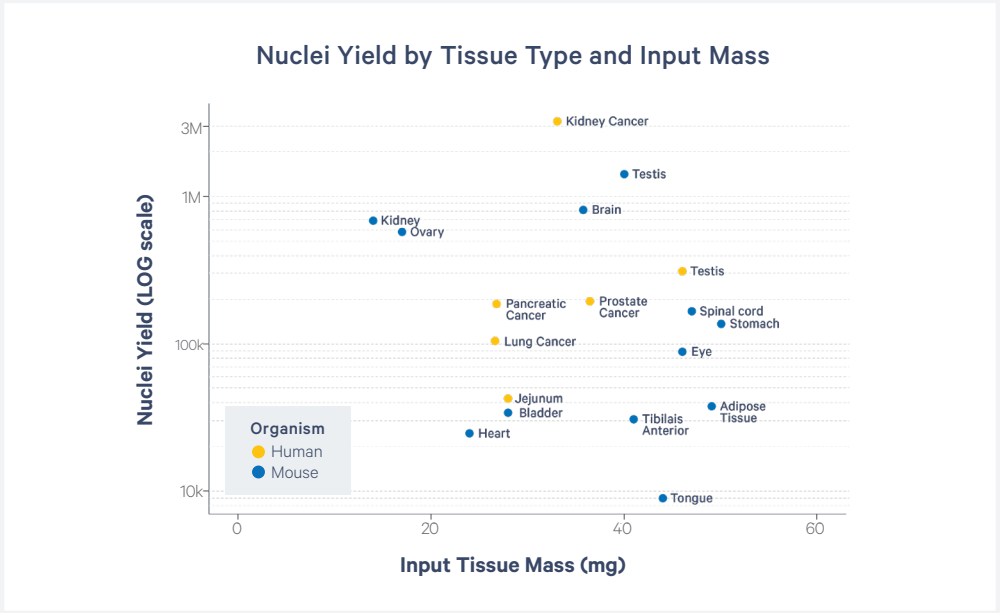
- After the isolation process, visualize nuclei suspensions to determine nuclei concentration and viability, suspension quality, and nuclei sizes prior to use in 10x Genomics Single Cell protocols.
- Counting with a fluorescent nucleic acid staining dye (e.g. AOPI or Ethidium Homodimer-1) and a fluorescent capable automated counter or microscope is strongly recommended as use of Trypan Blue can lead to overestimated nuclei counts.
- Vortex samples for **3 sec** immediately prior to counting to ensure an accurate estimate of the final nuclei concentration.
- Count nuclei in replicates (2–3 reproducible counts) to ensure accuracy.

Nuclei Concentration for Optimal Performance

- The total number of suspended nuclei used as input to 10x Genomics Single Cell protocols is determined by the nuclei recovery target. Consult the applicable 10x Genomics Single Cell protocol to determine these relationships.
- The optimal input nuclei concentration for most 10x Genomics Single Cell assays is **700–1,200 nuclei/µl**. For the Chromium Fixed RNA Profiling assay, a starting number of **1 x 10^6** nuclei is recommended. Refer to the relevant User Guide for specific information.
- If possible, bring the input nuclei suspension to a concentration that is optimal for the dynamic range of counting technique used (manual or automated), allows for 2–3 reproducible counts (where the standard deviation is <25%), and requires pipetting **2.5–15 µl** nuclei suspension (Single Cell 3' and 5' Gene Expression Assays) and **2–5 µl** (Single Cell Multiome ATAC + Gene Expression and Single Cell ATAC Assays) into the Single Cell Master Mix.
- Higher nuclei stock concentrations will result in lower pipetting volumes that may increase nuclei input variability.
- Lower nuclei stock concentrations will result in inaccurate nuclei counts that may also increase nuclei input variability.
- Use final single nuclei suspension to estimate the number of input nuclei since nuclei are inevitably lost during washing and resuspension steps.

Nuclei Recovery

- If using a limited sample and nuclei recovery is expected to be low, a single wash may be used in an attempt to improve recovery.
- In addition to a single wash, centrifugation time in the debris removal buffer and/or wash buffer may be extended to improve nuclei yield.
- The use of swinging-bucket rotors, which pellet nuclei to the bottom of tubes rather than the side, may also improve final nuclei recovery.



- The above graph shows that nuclei yield is impacted by starting tissue sample type. Nuclei recovery from healthy tissues typically ranges from **5,000–15,000 nuclei/mg**. Both disease state and cell type composition can impact nuclei recovery.
- Resuspend nuclei in a low initial volume **(50–200 µl)** during final resuspension step if nuclei yield is expected to be low or is unknown.
- DO NOT resuspend nuclei in a volume **<50 µl** regardless of input tissue mass.

Nuclei Control Sample

- The use of a quality control sample is recommended when using this protocol for the first time or testing new tissues types.
- Mouse brain tissue from BioIVT is a validated quality control sample and can be run in parallel with experimental samples. Additional validated tissues can be found in the list of Tested Tissues Types in the Appendix of this document.

Nuclei Storage

- Freezing or cryopreservation of nuclei following isolation is NOT recommended as the freezing and thawing process can damage the nuclear membrane and lead to loss of single-nuclei behavior. Additionally, frozen nuclei have the potential to burst and increase background signal.
- Cryopreserve at the tissue stage for best results in 10x Genomics Single Cell sequencing protocols.
- Nuclei can be stored after fixation for Chromium Fixed RNA Profiling applications for up to 1 week at **4°C** or for up to 3 months at **-20°C or -80°C**.

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Protocol Selector

Protocol Selector

The Nuclei Isolation protocol differs based on your desired downstream 10x Genomics Single Cell assay. The Chromium Nuclei Isolation Kit with RNase Inhibitor is compatible with Single Cell 3' Gene Expression, Single Cell 5' Gene Expression, Chromium Fixed RNA Profiling, and Single Cell Multiome + ATAC Gene Expression assays. The Chromium Nuclei Isolation Kit (without RNase Inhibitor) is compatible with Single Cell ATAC assays.

To ensure quality nuclei isolation preparation, proceed to the appropriate chapter outlined in the diagram below. Each chapter outlines the Reagent Kits, Get Started guidance, Buffer Preparation, and Protocol Steps for nuclei isolation of the selected Single Cell assay. The chapters are color coded with a labeled tab at the top of each page for easy navigation.

Note that following the appropriate nuclei isolation protocol is critical for the success of the nuclei isolation process as well as all downstream Single Cell applications.

Downstream Assay	Chapter and Page #s
Single Cell 3' and 5' Gene Expression *and* **Chromium Fixed RNA Profiling**	*Chapter 1:* **Nuclei Isolation Protocol for Single Cell Gene Expression & Chromium Fixed RNA Profiling** *for use with PN-1000494* Pages 25–32
Single Cell Multiome ATAC + Gene Expression	*Chapter 2:* **Nuclei Isolation Protocol for Single Cell Multiome ATAC + Gene Expression** *for use with PN-1000494* Pages 33–41
Single Cell ATAC	*Chapter 3:* **Nuclei Isolation Protocol for Single Cell ATAC** *for use with PN-1000493* Pages 42–50

10x GENOMICS

Nuclei Isolation Protocol:

Single Cell Gene Expression & Chromium Fixed RNA Profiling

Reagent Kits

Get Started

Buffer Preparation

Illustrative Overview

Nuclei Isolation Protocol Steps

Reagent Kits

Chromium Nuclei Isolation Kit with RNase Inhibitor PN-1000494

Chromium Nuclei Isolation Kits

Refer to SDS for handling and disposal information

For use with the following 10x Genomics products:

- Single Cell 3' Gene Expression
- Single Cell 5' Gene Expression
- Chromium Fixed RNA Profiling

Chromium
Nuclei Isolation Reagents
16 rxns, PN-1000447
Store at 4°C

	#	PN
Lysis Reagent	4	2000558
Surfactant A	1	2000559
Debris Removal Reagent	4	2000560

Chromium
Nuclei Isolation Consumables
16 rxns, PN-1000448
Ambient Temperature

	#	PN
Pestle	16	2000561
Nuclei Isolation Column	16	2000562
Collection Tube	16	2000563
Sample Dissociation Tube	16	2000564

Chromium
Reducing Agent B
PN-1000450
Store at -20°C

	#	PN
Reducing Agent B	1	2000087

Chromium
RNase Inhibitor
PN-1000449
Store at -20°C

	#	PN
RNase Inhibitor	5	2000565

Get Started

Nuclei Isolation Protocol:

Single Cell Gene Expression & Chromium Fixed RNA Profiling

If provided Lysis Reagent and Debris Removal Buffers appear cloudy or contain precipitate, warm the tubes to **40°C** *and swirl until the buffers become clear again.*

Action	Item	10x PN	Preparation & Handling	Storage
Place on Ice	**Lysis Reagent**	2000558	Vortex, verify no precipitate, and centrifuge briefly.	4°C
	Surfactant A	2000559	Vortex, verify no precipitate, and centrifuge briefly.	4°C
	Debris Removal Reagent	2000560	Vortex, verify no precipitate or layering, and centrifuge briefly.	4°C
	Reducing Agent B	2000087	Thaw to room temperature, vortex, verify no precipitate, and centrifuge briefly.	–20°C
	RNase Inhibitor	2000565	Centrifuge briefly.	–20°C
	Nuclei Isolation Consumables: • *Nuclei Isolation Column* • *Collection Tube*	2000562 2000563	Pre-chill assembled Nuclei Isolation Column(s) and Collection Tube(s) on ice.	Ambient
	Nuclease-free Water	—	See Buffer Preparation.	Ambient
	1X PBS	—	See Buffer Preparation.	Ambient
	10% BSA	—	See Buffer Preparation.	4°C
Place on Dry Ice	**Frozen Tissue Sample**	—	See Tips & Best Practices.	Liquid Nitrogen (long-term) or –80°C (short-term)
	Sample Dissociation Tube	2000564	Pre-chill on dry ice.	Ambient
Obtain	**Pestles**	2000561	Keep on lab bench.	Ambient
	Nucleic Acid Staining Fluorescent Dye	—	See Tips & Best Practices.	4°C
	Vortex	—	See Nuclei Isolation Protocol.	—

Buffer Preparation: Lysis Buffer & Debris Removal Buffer

Single Cell Gene Expression & Chromium Fixed RNA Profiling

Prepare the following Lysis and Debris Removal Buffers on ice shortly before starting the Nuclei Isolation protocol. Prepare large volumes in a 15-ml or 50-ml conical tube. Vortex briefly before use.

Lysis Buffer

Lysis Buffer (500 µl/rxn) *Add reagents in the order listed*	PN	1X+10% (µl)	4X + 10% (µl)	8X + 10% (µl)
Lysis Reagent	2000558	**550**	2,200	4,400
Reducing Agent B	2000087	**0.55**	2.2	4.4
Surfactant A	2000559	**5.5**	22	44
Total	–	**556.05**	2,224.2	4,448.4

Debris Removal Buffer

Debris Removal Buffer (500 µl/rxn) *Add reagents in the order listed*	PN	1X+10% (µl)	4X + 10% (µl)	8X + 10% (µl)
Debris Removal Reagent	2000560	**550**	2,200	4,400
Reducing Agent B	2000087	**0.55**	2.2	4.4
Total	–	**550.55**	2,202.2	4,404.4

Buffer Preparation: Wash and Resuspension Buffer

Single Cell Gene Expression & Chromium Fixed RNA Profiling

Prepare the following Wash and Resuspension Buffer on ice shortly before starting the Nuclei Isolation protocol. Prepare large volumes in a 15-ml or 50-ml conical tube. Vortex briefly before use.

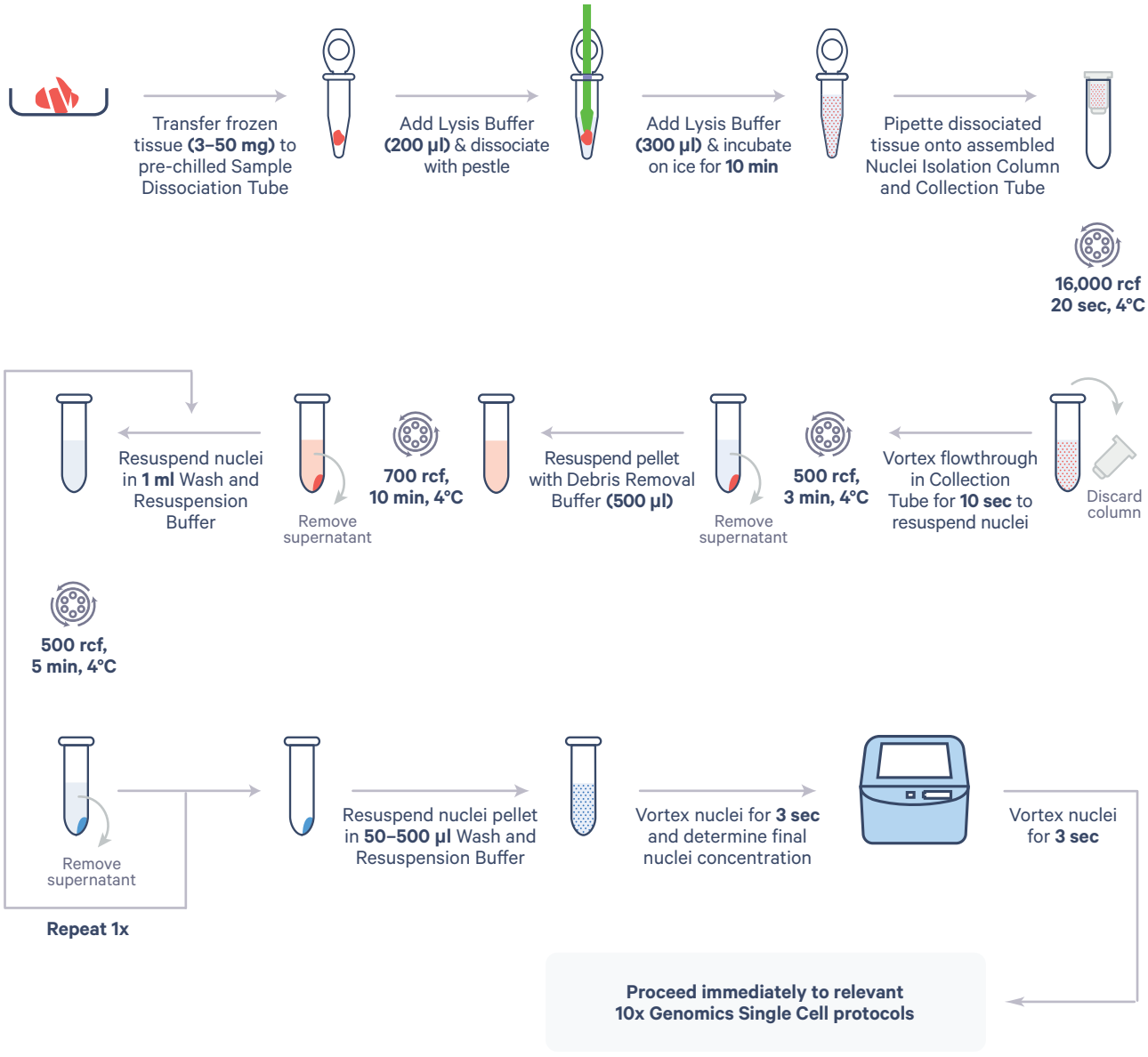
Wash and Resuspension Buffer

Wash and Resuspension Buffer (3 ml/rxn) *Add reagents in the order listed*	PN	1X+10% (µl)	4X + 10% (µl)	8X + 10% (µl)
1X PBS (not provided)	-	**2,887.5**	11,550	23,100
10% BSA (not provided)	-	**330**	1,320	2,640
RNase Inhibitor	2000565	**82.5**	330	660
Total	–	**3,300**	13,200	26,400

Protocol Overview: Nuclei Isolation from Frozen Tissue:

Single Cell Gene Expression & Chromium Fixed RNA Profiling

Nuclei Isolation



Nuclei Isolation Protocol:

Single Cell Gene Expression & Chromium Fixed RNA Profiling

a. 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.

b. Prepare Single Cell Gene Expression and/or Chromium Fixed RNA Profiling buffers according to Buffer Preparation section and place on ice.

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

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

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

f. 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.

g. 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.

h. Incubate on ice for **10 min**.

i. 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.

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

*Caps of tubes are angeled toward the balance tubes to prevent them from turning outward due to the centrifugal force.

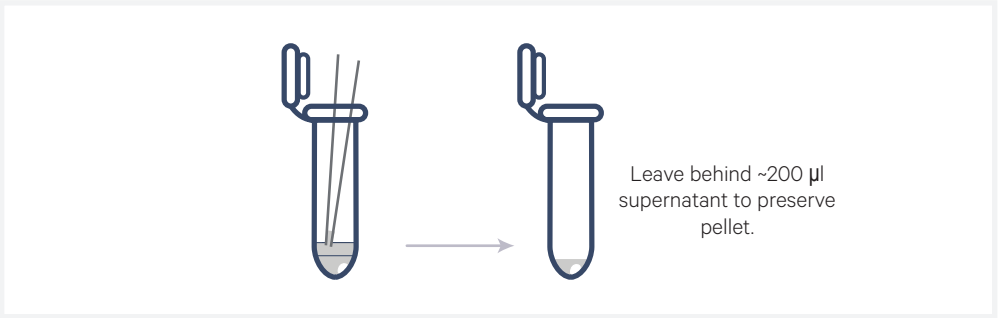
The absence of flowthrough following centrifugation indicates a clog in the column. Consult the Troubleshooting Guide for more information.

Nuclei Isolation Protocol:

Single Cell Gene Expression & Chromium Fixed RNA Profiling

k. 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.

l. 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.



TIPS *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.*

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

n. 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.

o. Resuspend nuclei pellet in **1 ml** of Wash and Resuspension Buffer.

p. 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.

q. Resuspend nuclei pellet in **1 ml** of Wash and Resuspension Buffer.

r. 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.

TIPS *For lower input tissue mass (<10 mg) or if low nuclei yield is expected, a single wash may be preferred to improve total nuclei yield.*

Nuclei Isolation Protocol:

Single Cell Gene Expression & Chromium Fixed RNA Profiling

s. Resuspend nuclei pellet in **50–500 µl** Wash and 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.

Resuspend in a low volume if nuclei yield is expected to be low or is unknown. DO NOT resuspend in a volume <50 µl.

t. 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.

u. 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.

v. 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.

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

10x GENOMICS

Nuclei Isolation Protocol:

Single Cell Multiome ATAC + Gene Expression

Reagent Kits

Get Started

Buffer Preparation

Illustrative Overview

Nuclei Isolation Protocol Steps

Reagent Kits

Chromium Nuclei Isolation Kit with RNase Inhibitor PN-1000494

Chromium Nuclei Isolation Kits

Refer to SDS for handling and disposal information

For use with the following 10x Genomics products:

- Single Cell Multiome ATAC + Gene Expression

Chromium
Nuclei Isolation Reagents
16 rxns, PN-1000447
Store at 4°C

	#	PN
Lysis Reagent	4	2000558
Surfactant A	1	2000559
Debris Removal Reagent	4	2000560

Chromium
Nuclei Isolation Consumables
16 rxns, PN-1000448
Ambient Temperature

	#	PN
Pestle	16	2000561
Nuclei Isolation Column	16	2000562
Collection Tube	16	2000563
Sample Dissociation Tube	16	2000564

Chromium
Reducing Agent B
PN-1000450
Store at -20°C

	#	PN
Reducing Agent B	1	2000087

Chromium
RNase Inhibitor
PN-1000449
Store at -20°C

	#	PN
RNase Inhibitor	5	2000565

Get Started

Nuclei Isolation Protocol:

Single Cell Multiome ATAC + Gene Expression

If provided Lysis Reagent and Debris Removal Buffers appear cloudy or contain precipitate, warm the tubes to **40°C** *and swirl until the buffers become clear again.*

Action	Item	10x PN	Preparation & Handling	Storage
Place on Ice	**Lysis Reagent**	2000558	Vortex, verify no precipitate, and centrifuge briefly.	4°C
	Surfactant A	2000559	Vortex, verify no precipitate, and centrifuge briefly.	4°C
	Debris Removal Reagent	2000560	Vortex, verify no precipitate or layering, and centrifuge briefly.	4°C
	Reducing Agent B	2000087	Thaw to room temperature, vortex, verify no precipitate, and centrifuge briefly.	–20°C
	RNase Inhibitor	2000565	Centrifuge briefly.	–20°C
	Nuclei Isolation Consumables: • *Nuclei Isolation Column* • *Collection Tube*	 2000562 2000563	Pre-chill assembled Nuclei Isolation Column(s) and Collection Tube(s) on ice.	Ambient
	20X Nuclei Buffer*	2000207	Thaw to room temperature, vortex, verify no precipitate, and centrifuge briefly.	–20°C
	Nuclease-free Water	—	See Buffer Preparation.	Ambient
	1X PBS	—	See Buffer Preparation.	Ambient
	10% BSA	—	See Buffer Preparation.	4°C
Place on Dry Ice	**Frozen Tissue Sample**	—	See Tips & Best Practices.	Liquid Nitrogen (long-term) or –80°C (short-term)
	Sample Dissociation Tube	2000564	Pre-chill on dry ice.	Ambient
Obtain	**Pestles**	2000561	Keep on lab bench.	Ambient
	Nucleic Acid Staining Fluorescent Dye	—	See Tips & Best Practices.	4°C
	Vortex	—	See Nuclei Isolation Protocol.	—

**20X Nuclei Buffer is included in the 10x Genomics Single Cell Multiome ATAC + Gene Expression Reagent Kits*

Buffer Preparation: Lysis Buffer & Debris Removal Buffer

Single Cell Multiome ATAC + Gene Expression

Prepare the following Lysis and Debris Removal Buffers on ice shortly before starting the Nuclei Isolation protocol. Prepare large volumes in a 15-ml or 50-ml conical tube. Vortex briefly before use.

Lysis Buffer

Lysis Buffer (500 µl/rxn) *Add reagents in the order listed*	PN	1X+10% (µl)	4X + 10% (µl)	8X + 10% (µl)
Lysis Reagent	2000558	**550**	2,200	4,400
Reducing Agent B	2000087	**0.55**	2.2	4.4
Surfactant A	2000559	**5.5**	22	44
Total	–	**556.05**	2,224.2	4,448.4

Debris Removal Buffer

Debris Removal Buffer (500 µl/rxn) *Add reagents in the order listed*	PN	1X+10% (µl)	4X + 10% (µl)	8X + 10% (µl)
Debris Removal Reagent	2000560	**550**	2,200	4,400
Reducing Agent B	2000087	**0.55**	2.2	4.4
Total	–	**550.55**	2,202.2	4,404.4

Buffer Preparation: Wash & Resuspension Buffers

Single Cell Multiome ATAC + Gene Expression

Prepare the following Wash and Resuspension Buffers on ice shortly before starting the Nuclei Isolation protocol. Prepare large volumes in a 15-ml or 50-ml conical tube. Vortex briefly before use.

Wash Buffer

Wash Buffer (2 ml/rxn) *Add reagents in the order listed*	PN	1X+10% (µl)	4X + 10% (µl)	8X + 10% (µl)
1X PBS (not provided)	-	**1,925**	7,700	15,400
10% BSA (not provided)	-	**220**	880	1,760
● **RNase Inhibitor**	2000565	**55**	220	440
Total	–	**2,200**	8,800	17,600

Resuspension Buffer

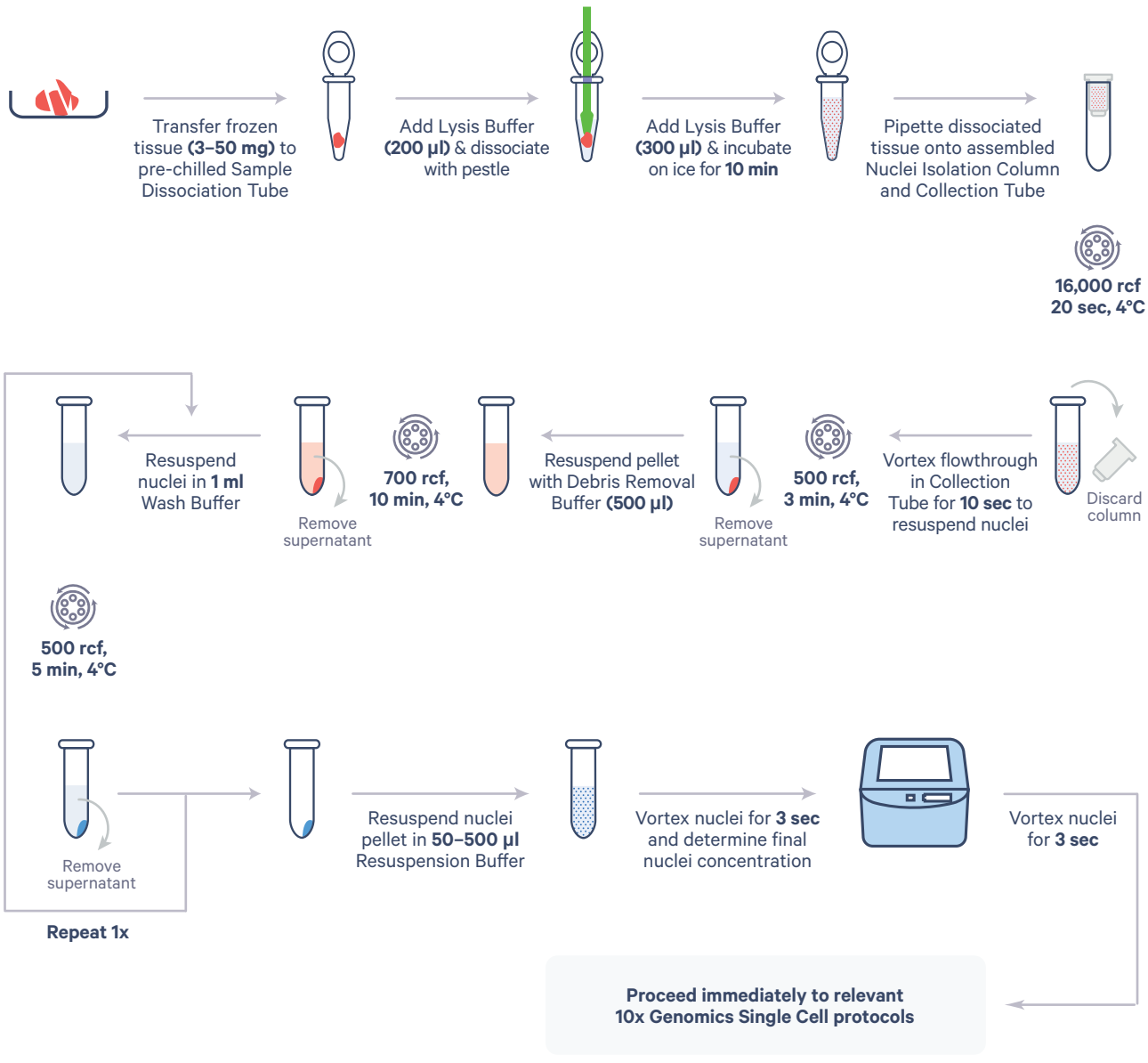
Resuspension Buffer (1 ml/rxn) *Add reagents in the order listed*	PN	1X+10% (µl)	4X + 10% (µl)	8X + 10% (µl)
● **20X Nuclei Buffer***	2000207	**55**	220	440
○ **Reducing Agent B**	2000087	**1.1**	4.4	8.8
Nuclease-free Water (not provided)	–	**1,016**	4,066	8,131
● **RNase Inhibitor**	2000565	**27.5**	110	220
Total	–	**1,099.6**	4,400.4	8,799.8

**20X Nuclei Buffer is included in the 10x Genomics Single Cell Multiome ATAC + Gene Expression Reagent Kits*

Protocol Overview: Nuclei Isolation from Frozen Tissue:

Single Cell Multiome ATAC + Gene Expression

Nuclei Isolation



Nuclei Isolation Protocol:

Single Cell Multiome ATAC + Gene Expression

a. 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.

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

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

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

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

f. 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.

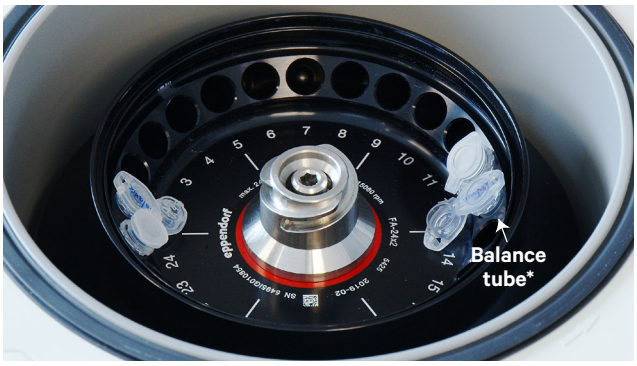
Perform tissue dissociation on ice. Use one pestle per sample. DO NOT discard pestles until nuclei isolation process is complete.

g. 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.

h. Incubate on ice for **10 min**.

i. 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.

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



*Caps of tubes are angeled toward the balance tubes to prevent them from turning outward due to the centrifugal force.

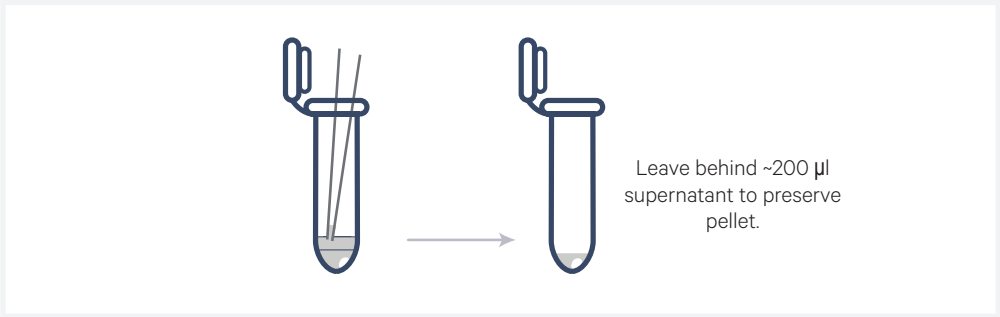
The absence of flowthrough following centrifugation indicates a clog in the column. Consult the Troubleshooting Guide for more information.

Nuclei Isolation Protocol:

Single Cell Multiome ATAC + Gene Expression

k. 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.

l. 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.



TIPS *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.*

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

n. 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.

o. Resuspend nuclei pellet in **1 ml** of Wash Buffer.

p. 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.

q. Resuspend nuclei pellet in **1 ml** of Wash Buffer.

r. 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.

TIPS *For lower input tissue mass (<10 mg) or if low nuclei yield is expected, a single wash may be preferred to improve total nuclei yield.*

Nuclei Isolation Protocol:

Single Cell Multiome ATAC + Gene Expression

s. 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.

Resuspend in a low volume if nuclei yield is expected to be low or is unknown. DO NOT resuspend in a volume <50 µl.

t. 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.

u. 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.

v. 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.

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

10x GENOMICS

Nuclei Isolation Protocol:

Single Cell ATAC

Reagent Kits

Get Started

Buffer Preparation

Illustrative Overview

Nuclei Isolation Protocol Steps

Chromium Nuclei Isolation Kit PN-1000493

For use with the following 10x Genomics products:

- Single Cell ATAC

Chromium
Nuclei Isolation Reagents
16 rxns, PN-1000447
Store at 4°C

	#	PN
Lysis Reagent	4	2000558
Surfactant A	1	2000559
Debris Removal Reagent	4	2000560

Chromium
Nuclei Isolation Consumables
16 rxns, PN-1000448
Ambient Temperature

	#	PN
Pestle	16	2000561
Nuclei Isolation Column	16	2000562
Collection Tube	16	2000563
Sample Dissociation Tube	16	2000564

Chromium
Reducing Agent B
PN-1000450
Store at -20°C

	#	PN
Reducing Agent B	1	2000087

Get Started

Nuclei Isolation Protocol:

Single Cell ATAC

If provided Lysis Reagent and Debris Removal Buffers appear cloudy or contain precipitate, warm the tubes to **40°C** *and swirl until the buffers become clear again.*

Action	Item	10x PN	Preparation & Handling	Storage
Place on Ice	**Lysis Reagent**	2000558	Vortex, verify no precipitate, and centrifuge briefly.	4°C
	Surfactant A	2000559	Vortex, verify no precipitate, and centrifuge briefly.	4°C
	Debris Removal Reagent	2000560	Vortex, verify no precipitate or layering, and centrifuge briefly.	4°C
	Reducing Agent B	2000087	Thaw to room temperature, vortex, verify no precipitate, and centrifuge briefly.	–20°C
	Nuclei Isolation Consumables: • *Nuclei Isolation Column* • *Collection Tube*	2000562 2000563	Pre-chill assembled Nuclei Isolation Column(s) and Collection Tube(s) on ice.	Ambient
	20X Nuclei Buffer*	2000207	Thaw to room temperature, vortex, verify no precipitate, and centrifuge briefly.	–20°C
	Nuclease-free Water	—	See Buffer Preparation.	Ambient
	1X PBS	—	See Buffer Preparation.	Ambient
	10% BSA	—	See Buffer Preparation.	4°C
Place on Dry Ice	**Frozen Tissue Sample**	—	See Tips & Best Practices.	Liquid Nitrogen (long-term) or –80°C (short-term)
	Sample Dissociation Tube	2000564	Pre-chill on dry ice.	Ambient
Obtain	**Pestles**	2000561	Keep on lab bench.	Ambient
	Nucleic Acid Staining Fluorescent Dye	—	See Tips & Best Practices.	4°C
	Vortex	—	See Nuclei Isolation Protocol.	—

**20X Nuclei Buffer is included in the 10x Genomics Single Cell ATAC v2 Reagent Kits*

Buffer Preparation: Lysis Buffer & Debris Removal Buffer

Single Cell ATAC

Prepare the following Lysis and Debris Removal Buffers on ice shortly before starting the Nuclei Isolation protocol. Prepare large volumes in a 15-ml or 50-ml conical tube. Vortex briefly before use.

Lysis Buffer

Lysis Buffer (500 µl/rxn) *Add reagents in the order listed*	PN	1X+10% (µl)	4X + 10% (µl)	8X + 10% (µl)
Lysis Reagent	2000558	**550**	2,200	4,400
Reducing Agent B	2000087	**0.55**	2.2	4.4
Surfactant A	2000559	**5.5**	22	44
PBS	–	**1,668**	6,673	13,345
Total	–	**2,225**	8,879	17,794

Debris Removal Buffer

Debris Removal Buffer (500 µl/rxn) *Add reagents in the order listed*	PN	1X+10% (µl)	4X + 10% (µl)	8X + 10% (µl)
Debris Removal Reagent	2000560	**550**	2,200	4,400
Reducing Agent B	2000087	**0.55**	2.2	4.4
Total	–	**550.55**	2,202.2	4,404.4

Buffer Preparation: Wash & Resuspension Buffers

Single Cell ATAC

Prepare the following Wash and Resuspension Buffers on ice shortly before starting the Nuclei Isolation protocol. Prepare large volumes in a 15-ml or 50-ml conical tube. Vortex briefly before use.

Wash Buffer

Wash Buffer (2 ml/rxn) *Add reagents in the order listed*	PN	1X+10% (µl)	4X + 10% (µl)	8X + 10% (µl)
1X PBS (not provided)	-	**1,980**	7,920	15,840
10% BSA (not provided)	-	**220**	880	1,760
Total	–	**2,200**	8,800	17,600

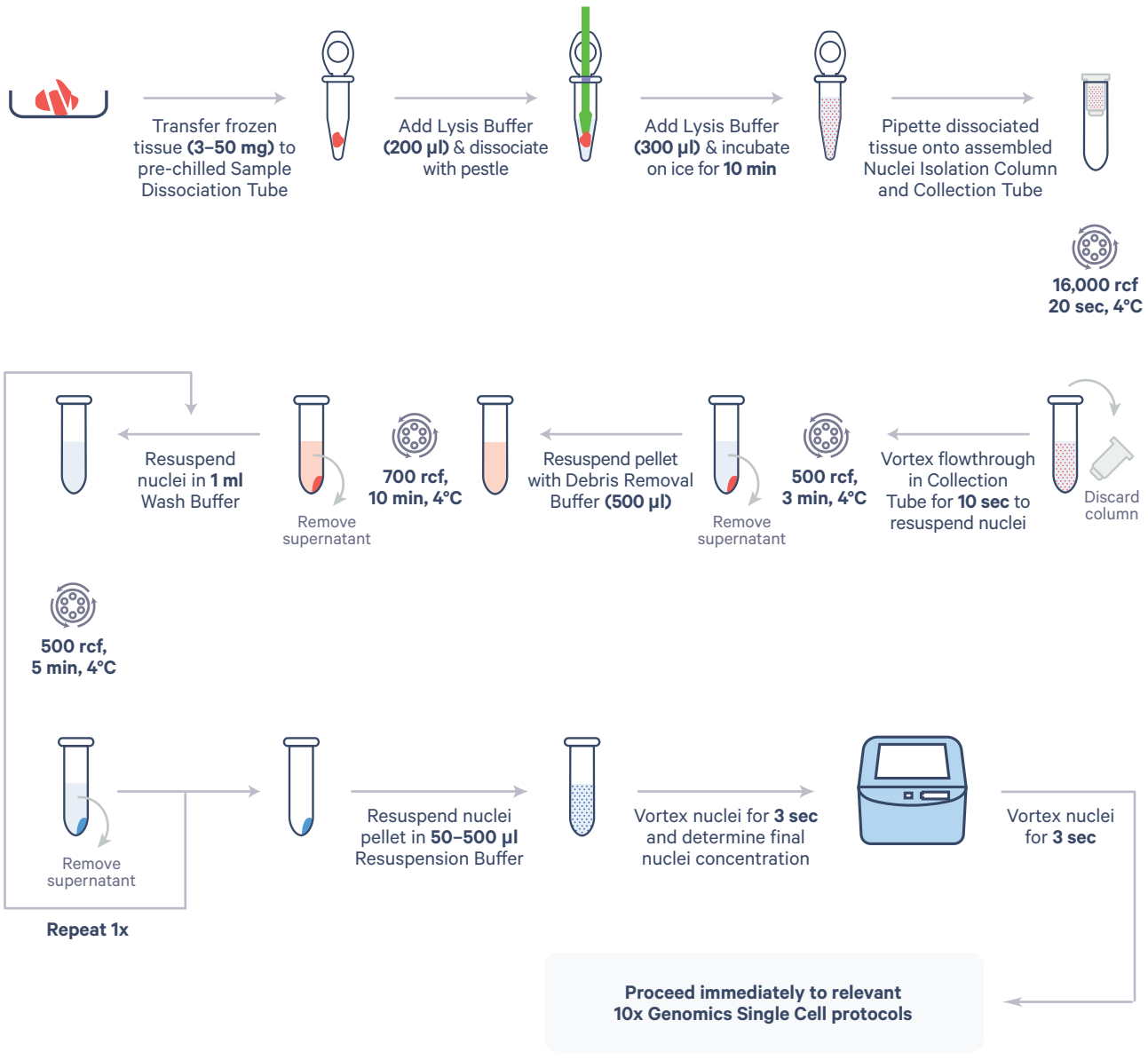
Resuspension Buffer

Resuspension Buffer (1 ml/rxn) *Add reagents in the order listed*	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 (not provided)	_	**1,044**	4,176	8,351
Total	–	**1,100.1**	4,400.4	8,799.8

**20X Nuclei Buffer is included in the 10x Genomics Single Cell ATAC v2 Reagent Kits*

Protocol Overview: Nuclei Isolation from Frozen Tissue:
Single Cell ATAC

Nuclei Isolation



Nuclei Isolation Protocol:

Single Cell ATAC

a. 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.

b. Prepare Single Cell ATAC buffers according to Buffer Preparation section and place on ice.

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

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

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

f. 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.

g. 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.

h. Incubate on ice for **10 min**.

i. 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.

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

*Caps of tubes are angeled toward the balance tubes to prevent them from turning outward due to the centrifugal force.

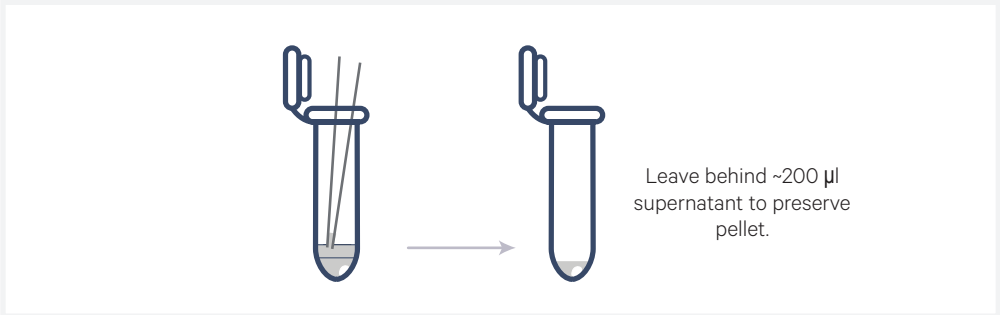
The absence of flowthrough following centrifugation indicates a clog in the column. Consult the Troubleshooting Guide for more information.

Nuclei Isolation Protocol:

Single Cell ATAC

k. 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.

l. 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.



TIPS *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.*

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

n. 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.

o. Resuspend nuclei pellet in **1 ml** of Wash Buffer.

p. 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.

q. Resuspend nuclei pellet in **1 ml** of Wash Buffer.

r. 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.

TIPS *For lower input tissue mass (<10 mg) or if low nuclei yield is expected, a single wash may be preferred to improve total nuclei yield.*

Nuclei Isolation Protocol:

Single Cell ATAC

s. 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.

Resuspend in a low volume if nuclei yield is expected to be low or is unknown. DO NOT resuspend in a volume <50 µl.

t. 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.

u. 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.

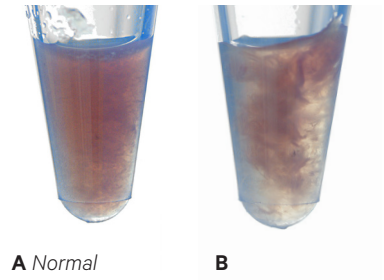
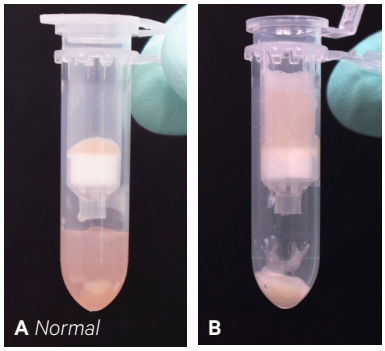
v. Vortex nuclei for **3 sec** at **3,200 rpm or max speed**. Pulse spin the tube after vortexing to collect liquid at bottom of tube. DO NOT pulse spin the tube for more than 1 second to ensure that nuclei do not pellet at the bottom of the tube.

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

10x GENOMICS

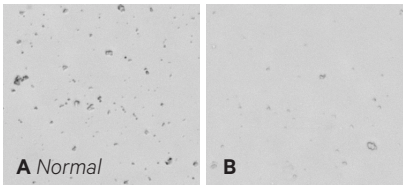
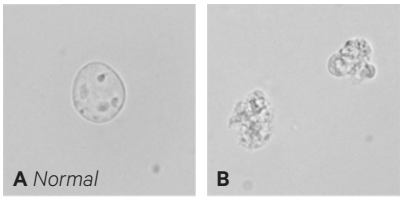
Troubleshooting

Troubleshooting Guide

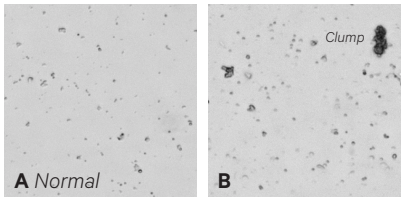
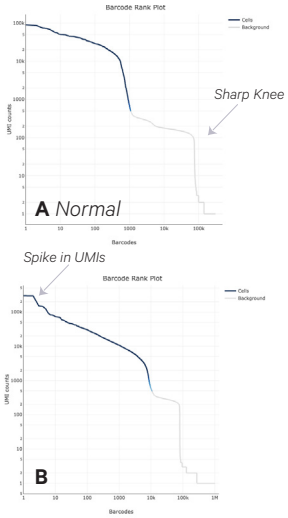
Issue	Potential Causes	Mitigation Strategies
Tissue not dissociated *(A) Dissociated tissue and (B) Tissue that is not fully dissociated*		
 **A** *Normal* **B**	• Tissue mass outside range of recommended tissue sizes. • Lysis time insufficient for dissociation. • Tissue type not compatible with Chromium Nuclei Isolation Kit. • Insufficient dissociation with pestle.	• Lyse tissue for a longer period of time. • Increase number of pestle strokes for complete tissue dissociation. • Ensure tissue mass is within recommended tissue size range.
Clogged nuclei isolation column *(A) Column with successful flowthrough and (B) Clogged column*		
 **A** *Normal* **B**	• Tissue mass outside range of recommended tissue sizes. • Tissue not fully dissociated. • Lysis time not sufficient for dissociation of tissue. • Tissue type not compatible with Chromium Nuclei Isolation Kit.	• Lyse tissue for a longer period of time. • Increase number of pestle strokes for complete tissue dissociation. • Ensure tissue mass is within recommended tissue size range. • Ensure tissue is not on list of Incompatible Tissue Types. • Use a wide-bore pipette to transfer tissues with excessive fibrous debris to limit tip clogging and maximize transfer of nuclei to the Nuclei Isolation Column. • In the event of a column clog, transfer liquid remaining on top of the Nuclei Isolation Column to a new column leaving behind any debris. Place into same Collection Tube and repeat centrifugation step.

If a column clog occurs, contact 10x Genomics Technical Support at support@10xgenomics.com for further assistance.

Troubleshooting Guide

Issue	Potential Causes	Mitigation Strategies
Low nuclei recovery *(A) Acceptable nuclei recovery and (B) Low nuclei recovery*		
	• Improper freezing and storage of input tissue. • Not enough starting tissue material. • Reagents and samples not kept on ice during Nuclei Isolation process. • Inaccurate counting of nuclei. • Tissue not fully dissociated. • Low cellularity of starting tissue. • Pellet lost or discarded during wash steps.	• Store tissue samples in liquid nitrogen for best results and at -80°C for short-term needs. • Minimize handling steps during tissue processing. • Perform entire Nuclei Isolation protocol on ice with chilled reagents and tools. • Reduce number of wash steps during Nuclei Isolation protocol. • Increase centrifugation times to 10 minutes during wash steps. • Use DNA-binding dye and a fluorescent microscope to count nuclei. • Use a swinging-bucket rotor centrifuge.
Poor nuclei quality *(A) Good nuclei quality and (B) poor nuclei quality with blebbing*		
	• Improper freezing and storage of input tissue. • Overlysis of nuclei. • Reagents and samples not kept on ice during Nuclei Isolation process. • Resuspension of nuclei is too harsh. • Excess delays between nuclei isolation and counting/loading of chip.	• Store tissue samples in liquid nitrogen for best results and at -80°C for short-term needs. • Minimize handling steps during tissue processing. • Perform entire Nuclei Isolation protocol on ice with chilled reagents and tools. • Minimize delays between start of lysis and loading of chip.

Troubleshooting Guide

Issue	Potential Causes	Mitigation Strategies
Debris and aggregates *(A) Acceptable and (B) unacceptable amounts of debris and aggregates*		
	• Overlysis of nuclei. • Cell lysis not performed on ice. • Resuspension between steps not sufficient. • Transfer of lysate to Debris Removal step without spin column filtration.	• Assess lysis efficacy via microscopy after incubation, if necessary. • Optimize lysis time for new tissues types or sizes. • Perform entire Nuclei Isolation protocol on ice. • Ensure sufficient vortex time and speed for resuspension. • If aggregates >50 µm are observed, the nuclei suspension may optionally be passed through a 40 µm FlowMi filter.*
Low cDNA/Poor sequencing data *(A) Normal barcode rank plot and (B) abnormal barcode rank plot*		
	• Improper freezing and storage of input tissue. • Samples not vortexed after resuspension steps. • Incorrect amount of RNase inhibitor added to buffers. • Samples degraded during isolation process.	• Store tissue samples in liquid nitrogen for best results and at -80°C for short-term needs. • Vortex samples where indicated during resuspension and wash steps. • Add the recommended amount of RNase inhibitor to buffers according to compatible assay, as outlined in the Buffer Preparation sections of this document. • Perform entire Nuclei Isolation protocol on ice. • Increase cDNA amplification by 1–2 cycles.

**Note that this additional filtration may cause a significant reduction in nuclei recovery.*

Appendix

Tested Tissue Types

Tissues with Variable Results

Optimization Recommendations

Compatible 10x Genomics Assays

Tested Tissue Types

Human tumors are highly complex tissues. Results can vary from sample to sample and within a single tumor biopsy.

The Chromium Nuclei Isolation Kit was optimized using human and mouse samples and is expected to be compatible with most mammalian tissues. The following tissues have been successfully tested using the Chromium Nuclei Isolation Kit within the supported mass range of the kit **(3–50 mg)**. Refer to the 10x Genomics Website for a complete and updated list of Tested Tissue Types.*

Organism	Tissue (Healthy/Tumor)
Mouse	Kidney (Healthy)
	Liver (Healthy)
	Lung (Healthy)
	Brain (Healthy)
	Heart (Healthy)
	Small Intestine (Healthy)
	Eye (Healthy)
	Skeletal Muscle (Healthy)
	Spinal Cord (Healthy)
	Bladder (Healthy)
	Ovary (Healthy)
	Colon (Healthy)
	Adipose (Healthy)
	Stomach (Healthy)
	Testis (Healthy)
Human	Jejunum (Healthy)
	Duodenum (Healthy)
	Ileum (Healthy)
	Testis (Healthy)
	Breast (Tumor)
	Prostate (Tumor)
	Melanoma (Tumor)

Tested Tissue Types *Contd.*

Human tumors are highly complex tissues. Results can vary from sample to sample and within a single tumor biopsy.

Organism	Tissue (Healthy/Tumor)
Human	Ovarian (Tumor)
	Colorectal (Tumor)
	Pancreas (Tumor)
	Kidney (Tumor)
	Lung (Tumor)

**Note that tissues not listed may still be compatible with the Chromium Nuclei Isolation Kit. This list only summarizes tissues validated specifically by 10x Genomics.*

Tissues with Variable Results

The following tissues may require further optimization for use with the Chromium Nuclei Isolation Kit. Note that tissues with high RNase content are sensitive to collection and storage conditions. Refer to Tip & Best Practices for guidance on tissue handling and storage. Cut tissues with lower yields into small pieces (≤10 mg) before dissociation in Lysis Buffer. See Tissue Dissociation section of Tips & Best Practices for more information.

Organism	Tissue (Healthy/Tumor)	Additional Notes
Mouse	Pancreas (Healthy)	High RNase tissue
	Spleen (Healthy)	High RNase tissue
	Skin (Healthy)	Lower yield tissue
	Tongue (Healthy)	Lower yield tissue
Human	Spleen (Healthy)	High RNase tissue

Incompatible Tissue Types

The following tissues have been tested by 10x Genomics and are NOT recommended for use with the Chromium Nuclei Isolation Kit:

- Cell suspensions (i.e. cultured cells, PBMCs)
- Plants
- Insects
- Calcified tissue (i.e. bone)
- FFPE tissue

Optimization Recommendations

The Chromium Nuclei Isolation Kit protocol was validated without modification using the indicated Tested Tissue Types. If expected performance is not achieved for tissue(s) of interest using the recommended protocol, the following optimization of some protocol steps may improve performance based on the unique properties of target tissue(s).

Lysis time:

- Perform a lysis timeline to determine appropriate lysis incubation time for new tissue types.

Lysis buffer strength:

- If nuclei quality is poor, buffer detergent strength can be decreased for a gentler lysis by diluting with 1X PBS.

Sample cleanup steps:

- Additional washes may further reduce small debris and ambient RNA in the sample.

Compatible 10x Genomics Assays

Reagent Kit/User Guide	Document Number
Single Cell 3' Gene Expression	
Chromium Next GEM Single Cell 3' HT Reagent Kits User Guide (v3.1 - Dual Index)	CG000416
Chromium Next GEM Single Cell 3' Reagent Kits User Guide (v3.1 - Dual Index)	CG000315
Chromium Next GEM Single Cell 3' Reagent Kits User Guide (v3.1 - Single Index)	CG000204
Chromium Next GEM Single Cell 3' LT Reagent Kits User Guide (v3.1 - Dual Index)	CG000399
Single Cell 5' Gene Expression	
Chromium Next GEM Single Cell 5' HT Reagent Kits User Guide (v2 - Dual Index)	CG000423
Chromium Single Cell 5' Reagent Kits User Guide (v2 - Dual Index)	CG000331
Single Cell ATAC	
Chromium Single Cell ATAC Reagent Kits User Guide (v2 Chemistry)	CG000496
Single Cell Multiome ATAC + Gene Expression	
Chromium Next GEM Single Cell Multiome ATAC + Gene Expression Reagent Kits User Guide	CG000338
Chromium Fixed RNA Profiling	
Chromium Fixed RNA Profiling Reagent Kits for Singleplexed Samples with Feature Barcode technology for Cell Surface Protein User Guide	CG000477
Chromium Fixed RNA Profiling Reagent Kits for Multiplexed Samples User Guide	CG000527