Jun 18, 2024

Nuclei Isolation from Frozen Tissue or Frozen hPCLS

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.eq2lyw6ewvx9/v1

Heidi Monroe[1], Nayra Cardenes[2], Melanie Königshoff[2], koenigshoffm[2], Robert Lafyatis[1]

[1]University of Pittsburgh;
[2]Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, School of Medicine, University of Pittsburgh

DOI: dx.doi.org/10.17504/protocols.io.eq2lyw6ewvx9/v1

Protocol Citation: Heidi Monroe, Nayra Cardenes, Melanie Königshoff, koenigshoffm, Robert Lafyatis 2024. Nuclei Isolation from Frozen Tissue or Frozen hPCLS. **protocols.io https://dx.doi.org/10.17504/protocols.io.eq2lyw6ewvx9/v1**

Protocol status: Working

Created: June 12, 2024

Last Modified: June 18, 2024

Protocol Integer ID: 101647

Keywords: Nuclei isolation, Frozen tissue, snRNAseq, PCLS, Chromium, Lung, SenNet, TriState

Funders Acknowledgement:
TriState SenNET (Lung and Heart) Tissue Map and Atlas consortium - NIA
Grant ID: U54AG075931

Abstract

This protocol follows the "Chromium Nuclei Isolation Kit" guidelines for the process for isolating Nuclei from frozen tissues and/or PCLS (Precision-Cut Lung Slices) for use in compatible 10x Genomics Single Cell assays.

Attachments

DOCX

snRNAseq_ProtocolsIO..

66KB

Image Attribution

Nayra Cardenes, PhD

Materials

Buffer preparation:

Lysis Buffer & Debris Removal Buffer:

	A	B	C	D
	Lysis Buffer (500 µl/rxn) Add reagents in the order listed	1X+10% (µl)	4X+10% (µl)	8X+10% (µl)
	Lysis Reagent	550	2,200	4,400
	Reducing Agent B	0.55	2.2	4.4
	Surfactant A	5.5	22	44
	Total	556.05	2,224.20	4,448.40

Calculations for Lysis Buffer Preparation.

Debris Removal Buffer:

	A	B	C	D
	Debris Removal Buffer (500 µl/rxn) Add reagents in the order listed	1X+10% (µl)	4X+10% (µl)	8X+10% (µl)
	Debris Removal Reagent	550	2,200	4,400
	Reducing Agent B	0.55	2.2	4.4
	Total	550.55	2,202.20	4,404.40

Calculations for Removal Buffer Preparation.

Wash and Resuspension Buffer:

	A	B	C	D
	Wash and Resuspension Buffer (3 ml/rxn) Add reagents in the order listed	1X+10% (µl)	4X+10% (µl)	8X+10% (µl)
	1X PBS	2,887.50	11,550	23,100
	10% BSA	330	1,320	2,640
	RNase Inhibitor	82.5	330	660
	Total	3,300	13,200	26,400

Calculations for Wash and Resuspension Buffer Preparation.

Equipments:

Sample Dissociation Tube **10x Genomics Catalog #**2000564

Nuclei Isolation column **10x Genomics Catalog #**2000562

Collection Tube **10x Genomics Catalog #**2000563

Chromium Nuclei Isolation Kit with RNase Inhibitor **10x Genomics Catalog #**PN-1000494

Before start

Note

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.

- **Pre-chill centrifuge to** 4 °C
- **Thaw Reducing Agent B** – Thaw to Room temperature .
- **Vortex** – Vortex, verify no precipitate, and centrifuge briefly all Lysis and Debris Removal reagents,
- **RNase Inhibitor** – Centrifuge briefly.
- **Buffer Preparation: Lysis Buffer & Debris Removal Buffer** - 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.
- **Buffer Preparation: Wash and Resuspension Buffer** - 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.
- **Place reagents and tubes on ice** – Label tops and sides of tubes, as well as tops of spin columns, before placing On ice and starting protocol.
- **Place Tissue and sample dissociation tubes on dry ice** – Pre-chill on dry ice.

Nuclei Isolation

28m 36s

1 Prepare all buffers in advance.

2

> **Note**
>
> **Perform all protocol steps** On ice **and centrifugation steps at** 4 °C .

Transfer frozen tissue (~ 50 mg ; use 2 slices if isolating from PCLS) to pre-chilled Sample Dissociation Tube (2000564) and place on wet ice.

3 Add lysis buffer (200 µL) & dissociate with pestle until homogeneous while On ice .

4

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

Add lysis buffer (300 µL) and pipette mix 10×. If not homogeneous, continue to dissociate with the pestle until able to pipette mix.

5 Incubate On ice for 00:10:00 .

10m

6 Pipette dissociated tissue onto assembled and pre-chilled Nuclei Isolation Column and Collection Tube (2000562 & 2000563).

7 Centrifuge at 16000 rcf, 4°C, 00:00:20 .

20s

8 Discard column.

9 Vortex flowthrough in Collection Tube for 3200 rpm, 00:00:10 minimum to resuspend nuclei. 10s

10 Centrifuge at 500 rcf, 4°C, 00:03:00 . 3m

11 Remove supernatant (s/n).

12 Resuspend pellet with debris removal buffer (500 µL).

13 Centrifuge at 700 rcf, 4°C, 00:10:00 . 10m

14 Remove supernatant (s/n).

15 Resuspend nuclei in 1 mL wash and resuspension Buffer.

16 Centrifuge at 500 rcf, 4°C, 00:05:00 . 5m

17 Remove supernatant (s/n).

18 Repeat 15-17

19 Resuspend nuclei pellet in 50 µL – 500 µL wash and resuspension Buffer.

20 Vortex nuclei for 00:00:03 and determine final nuclei concentration using AOPI or Ethidium Homodimer-1 fluorescent staining dyes and dilute if necessary for target nuclei load. Adjust nuclei concentration as necessary for intended downstream assay. 3s

21 Vortex nuclei for 00:00:03 and keep samples On ice . 3s

Note

Proceed immediately to 10× Genomics Single GEM Generation and Barcoding.

Protocol references

https://cdn.10xgenomics.com/image/upload/v1660261285/support-documents/CG000505_Chromium_Nuclei_Isolation_Kit_UG_RevA.pdf