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Nuclei Isolation from Frozen Tissue or Frozen hPCLS



In 1 collection

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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



snRNAseq_ProtocolsIO..

66KB

Image Attribution

Nayra Cardenes, PhD



Materials

Buffer preparation:

Lysis Buffer & Debris Removal Buffer:

| A | В | С | 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 | | В | С | D |
|---------------------------------------|--|-------------|-------------|-------------|
| Debris Removal E reagents in the o | Buffer (500 µl/rxn) Add rder listed | 1X+10% (μl) | 4X+10% (μl) | 8X+10% (μl) |
| Debris Removal F | Reagent | 550 | 2,200 | 4,400 |
| Reducing Agent I | В | 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 | В | С | D |
|--|-------------|-------------|-------------|
| Wash and Resuspension Buffer (3 ml/rxn) Add reagents in the order listed | 1X+10% (µl) | 4X+10% (μl) | 8X+10% (µI) |
| 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 B Room temperature I.
- 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 If 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 If 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.

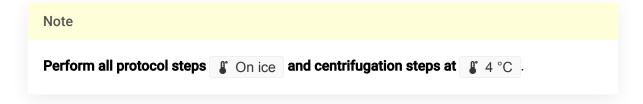


Nuclei Isolation

28m 36s

1 Prepare all buffers in advance.

2



Transfer frozen tissue (~ 4 50 mg); use 2 slices if isolating from PCLS) to pre-chilled Sample Dissociation Tube (2000564) and place on wet ice.

4

Note

Add lysis buffer ($\underline{\bot}$ 300 μ L) and pipette mix 10×. If not homogeneous, continue to dissociate with the pestle until able to pipette mix.

- 5 Incubate 6 On ice for 00:10:00 .
- 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

10m





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

10 Centrifuge at \$\infty\$ 500 rcf, 4°C, 00:03:00 .

3m

- 11 Remove supernatant (s/n).
- 12 Resuspend pellet with debris removal buffer (\$\rm 500 \mu L\$).
- 13



- 14 Remove supernatant (s/n).
- 15 Resuspend nuclei in 🚨 1 mL wash and resuspension Buffer.
- 16 Centrifuge at \$\mathref{\math



- 17 Remove supernatant (s/n).
- 18 Repeat 15-17
- 19 Resuspend nuclei pellet in Δ 50 μ L - Δ 500 μ L wash and resuspension Buffer.



20 Vortex nuclei for 00:00:03 and determine final nuclei concentration using AOPI or 3s Ethidium Homodimer-1 fluorescent staining dyes and dilute if necessary for target nuclei load. Adjust nuclei concentration as necessary for intended downstream assay. 21 Vortex nuclei for 00:00:03 and keep samples 00 ice. 3s Note Proceed immediately to 10× Genomics Single GEM Generation and Barcoding.

Protocol references

https://cdn.10xgenomics.com/image/upload/v1660261285/supportdocuments/CG000505_Chromium_Nuclei_Isolation_Kit_UG_RevA.pdf