Nuclei isolation from mouse lung for single nucleus RNASeq

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Protocol status: Working
 We use this protocol and it's working

Nuclei isolation from mouse lung for single nucleus RNASeq

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Human Cell Atlas Method Development Community

Jeffrey Koenitzer

ABSTRACT

This protocol is for **nuclei isolation from mouse lung for single nucleus RNASeq**.

It is adapted directly from Joshi et al., with adjustments to RNase inhibitor concentrations and removal of FACS sorting steps.

CITATION

Nikita Joshi, Alexander Misharin. Single-nucleus isolation from frozen human lung tissue for single-nucleus RNA-seq.

LINK

dx.doi.org/10.17504/protocols.io.zu8f6zw

ATTACHMENTS

Protocol for nuclei isolation from mouse lung for single nucleus RNASeq.pdf

step-by-step_Lung nuclear isolation protocol v1.pdf

MATERIALS

MATERIALS

Created: Mar 18, 2020

Last Modified: Apr 07, 2020

PROTOCOL integer ID:
34458

Keywords: nuclei isolation,
mouse, mouse lung, RNASeq,
single nucleus RNASeq

✕ RNaseZap® Thermo Scientific Catalog #AM9780

✕ cOmplete ULTRA Tablets, Mini, EDTA-free, EASYpack Roche Catalog #05 892 79 001

✕ RNasin Plus Ribonuclease Inhibitors Promega Catalog #N2615

✕ SUPERaseIN RNase Inhibitor Thermo Fisher Scientific Catalog #AM2696

✕ RNase free H2O Thermo Scientific Catalog
#AM9938

✕ Albumin, Bovine Serum, 10% Aqueous Solution, Nuclease-Free Millipore
Sigma Catalog #126615-25ML

✕ Gibco™ DPBS no calcium no magnesium Thermo Fisher Scientific Catalog
#14190144

✕ Nuclei Isolation Kit: Nuclei EZ Prep Sigma Aldrich Catalog #NUC-
101

Note

The *Nuclei Isolation Kit* contains the *Nuclei EZ Lysis Buffer* that is required for the preparation of Lysis Buffer and cOmplete stock (10x).

Storage Conditions

Material	Storage
1x DPBS	4 °C
Nuclei EZ Lysis Buffer (from kit NUC-101)	4 °C
cOmplete ULTRA Tablet s, Mini, EDTA-free, EASY pack	4 °C

RNasin Plus Ribonuclease Inhibitors	-20 °C
SUPERase RNase Inhibitor	-20 °C
RNase free H2O	RT
RNaseZap	RT
Bovine serum albumin, 10% solution, nuclease free	-20 °C

Equipment

gentleMACS™ C Tubes

NAME

Tissue dissociators and tubes

TYPE

gentleMACS™

BRAND

130-093-237

SKU

<https://www.miltenyibiotec.com/AD-en/products/macs-sample-preparation/tissue-dissociators-and-tubes/c-tubes/gentlemacs-c-tubes.html>

LINK

Equipment	
gentleMACS™ Dissociator	NAME
Tissue Dissociator	TYPE
MACS	BRAND
130-093-235	SKU
https://www.miltenyibiotec.com/US-en/products/macs-sample-preparation/tissue-dissociators-and-tubes/gentlemacs-dissociator/gentlemacstm-dissociator.html	LINK

Equipment	
pluriStrainer® 40 µm	NAME
Cell Strainer	TYPE
pluriSelect	BRAND
43-50040	SKU
https://www.pluriselect.com/pluristrainer-40-um-cell-strainer-51.html	LINK

Equipment	
pluriStrainer® 5 µm	NAME
Cell Strainer	TYPE
pluriSelect	BRAND
43-50005	SKU
https://www.pluriselect.com/eu/pluristrainerr-5-m-cell-strainer.html#variation=150	LINK

Equipment	
C-Chip™ Disposable Hemacytometers (Fuchs Rosenthal)	NAME
Counting Chamber	TYPE
INCYTO	BRAND
DHCF015	SKU
https://us.vwr.com/store/product/4618278/incyto-c-chiptm-disposable-hemacytometers-sk	LINK

Equipment	
Falcon™ 15mL Polystyrene Conical Centrifuge Tubes	NAME
Centrifuge Tubes	TYPE
Falcon™	BRAND
352095	SKU
https://www.fishersci.com/shop/products/falcon-15ml-conical-centrifuge-tubes-5/p-193301	LINK

Equipment	
Ambion® RNase-free 50 ml Conical Tubes	NAME
Centrifuge Tubes	TYPE
Ambion®	BRAND
AM12502	SKU
https://www.thermofisher.com/order/catalog/product/AM12502#/AM12502	LINK

Equipment	
TPP 60 mm Tissue Culture Dishes	NAME
Tissue Culture Dish	TYPE
TPP	BRAND
TP93060	SKU
https://midsci.com/item/ASTPPDISH/TPP-Dishes/	LINK

SAFETY WARNINGS

Please see SDS (Safety Data Sheet) for hazards and safety warnings.

BEFORE START INSTRUCTIONS

Prepare Working Solutions

Complete stock (10x)

- 1 mL Nuclei EZ Prep lysis buffer
- 1 tablet cOmplete ULTRA tablets

Lysis Buffer - 2 ml per $< 6 \text{ mm}^3$ tissue

- 200 μL cOmplete stock
- 1.775 mL Nuclei EZ Prep lysis buffer
- 12.5 μL RNasin Plus
- 12.5 μL SUPERaseIN

Wash Buffer - 4 ml per $< 6 \text{ mm}^3$ tissue

- 3.575 mL dPBS
- 400 μL 10% BSA
- 12.5 μL RNasin Plus
- 12.5 μL SUPERaseIN

Resuspension Buffer – 1 ml

- 0.977 mL dPBS
- 10 μL 10% BSA
- 6.25 μL RNasin Plus
- 6.25 μL SUPERaseIN

1 Pre-cool all instruments (including centrifuges), buffers, and tubes.

Note

Note: All steps are performed On ice or in cold room (4°C) to minimize RNA degradation.

- 2 Remove mouse lung sample from -80 °C freezer and trim a **~6 mm³ piece**.
- 3 Thaw on small plastic weighing boat until able to insert 26G needle to tissue, then inject ice cold **1 mL Lysis Buffer** to 'inflate' the tissue. Add the remaining Lysis Buffer and chop to the smallest pieces possible with scissors (60 s).
- 4 Transfer the minced tissue and buffer to a GentleMacs C tube.
- 5 Close, invert, and transfer directly to MACS Tissue dissociator.
- 6 Run *m_lung_01 program* and *m_lung_02 program* in sequence, stopping the latter after **00:00:20**.

Foam after GentleMacs

7 Place tube On ice .

8 Reduce foam by centrifugation (750 x g, 00:01:00).

Foam after centrifugation

- 9 Using a wide bore tip, pipette up and down to recover any pelleted material and pass lysate through 40 μ m filter to 50 ml conical tube.

- 10 Wash strainer with 4 mL Wash Buffer

- 11 Pass suspension through a 5 μ m strainer into 50 ml conical tube.

- 12 Centrifuge at 600 x g, 4°C, 00:05:00

13

Resuspend in

500 μ L Resuspension
Buffer

14

Count nuclei by hemocytometer and dilute to desired concentration (e.g. 10,000 per μ l).

Note

Note: it is easier to resuspend in lower volumes and dilute than to concentrate nuclei via further centrifugation.

40x Nuclear suspension with scant debris

15

Proceed to 10x Chromium.