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Harvesting cells for single-cell RNA-sequencing

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Protocol status: Working

We use this protocol and it's working

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Keywords: single cell rna, harvesting cell, samples from mouse lung tissue, cell rna, scrna, mouse lung tissue, rna, cell, sequencing protocol, sequencing, extracting sample

Abstract

Protocol for extracting samples from mouse lung tissue and then harvesting cells to be used in scRNA-seq.

Guidelines

Research involving animals must be conducted according to internationally-accepted standards and should always have prior approval from the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee.

Protocol materials

⊗ Pentobarbital sodium **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Y0002194**

⊗ HBSS **Gibco - Thermo Fisher Scientific Catalog #14060040**

⊗ Dispase **Corning Catalog #354235**

⊗ deoxyribonuclease I (DNase I, 100 U/ml) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D 4263**

⊗ BD Pharm Lyse **BD Biosciences Catalog #555899**

⊗ DMEM **Invitrogen - Thermo Fisher**

⊗ Fetal Bovine Serum (FBS) **ATCC Catalog #30-2020**

⊗ Acridine orange **Fisher Scientific Catalog #AC423340010**

⊗ Propidium iodide



- 1 Mice were euthanized with
 Pentobarbital sodium **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Y0002194**
- 2 The chest cavity was opened and lungs were perfused through the right ventricle with
 10 mL  HBSS **Gibco - Thermo Fisher Scientific Catalog #14060040**
- 3 The lungs were removed and, using a  30 g needle, infused with  1 mL
 Dispase **Corning Catalog #354235** with
 deoxyribonuclease I (DNase I, 100 U/ml) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D 4263**
- 4 The lungs were incubated at room temperature with gentle agitation for  00:45:00
- 5 The lungs were gently teased into small ( 1-2 mm) fragments
- 6 The lungs were incubated in a digestion buffer for another  00:15:00
- 7 The resulting suspension was passed through a 70 µm cell strainer and washed with DMEM supplemented with 5% FBS
- 8 The resulting suspension was pelleted by centrifugation
- 9 Erythrocytes were lysed using  BD Pharm Lyse **BD Biosciences Catalog #555899**
- 10 The resulting single-cell suspension was kept in
 DMEM **Invitrogen - Thermo Fisher** and
 Fetal Bovine Serum (FBS) **ATCC Catalog #30-2020** and passed through a



Equipment

40 mm cell strainer

NAME

Falcon

BRAND

352340

SKU

<https://ecatalog.corning.com/life-sciences/b2c/US/en/Cell-Culture/Cell-Culture-Accessories/Cell-Strainers/Falcon%C2%AE-Cell-Strainers/p/352340>

LINK

two times

11 Cells were counted using a

Equipment

K2 Fluorescent Cell Counter

NAME

Cell Counter

TYPE

Cellometer

BRAND

CMT-K2-MX-150

SKU

<https://www.revvity.com/ie-en/product/cellometer-k2-bundle-w-matrix-software-cmt-k2-mx-150>

LINK

with nucleic acid binding dyes

 Acridine orange **Fisher Scientific Catalog #AC423340010** to calculate total number of nucleated cells and  Propidium iodide to count dead cells