Mouse Lung Digestion to Single-Cell Suspension

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Purpose

This protocol describes the enzymatic and mechanical dissociation of mouse lung tissue to obtain single-cell suspensions suitable for flow cytometry, FACS sorting, and single-cell sequencing. Lung tissue is fibrous and elastic; therefore, digestion requires both **collagenase** and **DNase** together with thorough mechanical disruption.

Critical notes (read before starting)

- Keep all buffers and tubes **cold** except during digestion at **37 °C**.
- Use **fresh, pre-warmed digestion buffer** with DNase added on the day of use.
- Lung tissue contains **trapped blood**; incomplete perfusion increases RBC contamination.
- Do not over-digest: limit digestion to **30–35 minutes**, as prolonged incubation reduces viability.
- After digestion, perform all downstream steps on **ice**.
- Use **70 m strainers** and do not reuse between lungs.

Approximate timing

- Dissection: **5 min**
- Mince + pre-digestion handling: **5 min**
- Enzymatic digestion: **30–35 min**
- Mechanical processing and washes: **20–25 min**

Total hands-on time: ~**60 minutes per lung** (less when batching).

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Procedure

STEP 1 – Lung collection

1. Euthanise the mouse using an approved method.
2. Open the thoracic cavity to expose the lungs.
3. (Optional but recommended) Perfuse via the right ventricle with **cold PBS** until lungs visibly blanch, reducing RBC content.
4. Excise the entire lung block and place it in a **10 cm petri dish** containing **cold PBS or FACS/MACS Buffer**.

> Tip
>
> Perfusion markedly improves final leukocyte purity and reduces RBC lysis requirements.

STEP 2 – Tissue mincing

1. Blot the lungs briefly on sterile tissue to remove excess fluid.
2. Transfer to a fresh **10 cm dish** and mince thoroughly using scissors or scalpels until small fragments are obtained.
3. Transfer the minced lung into a **50 mL Falcon tube** containing **5 mL Lung Digestion Buffer** (see buffer protocol `BUF-LNG-DIG-v1.0`).

STEP 3 – Enzymatic digestion

1. Place tubes on a **37 °C benchtop shaker** at **250 rpm**.
2. Incubate for **30–35 minutes**.

> Warning
>
> Over-digestion can decrease viability and negatively impact epithelial and myeloid populations.

STEP 4 – Mechanical dissociation

1. After digestion, add **FACS/MACS Buffer** to bring total volume to **25 mL**.
2. Pour the suspension onto a **70 m cell strainer** placed on a **10 cm dish**.
3. Mechanically mash the digested lung through the mesh using the **back of a 5 mL syringe plunger**.
4. Rinse the strainer with **cold FACS/MACS Buffer** until no visible tissue remains.
5. Transfer the filtered cell suspension back into a **50 mL tube**.
6. Top up to **50 mL** with cold FACS/MACS Buffer.
7. Centrifuge at **400 g for 5 minutes at 4 °C**.
8. Carefully discard the supernatant.

STEP 5 – RBC removal (if required)

Lung digests vary in RBC content. If erythrocytes are visible:

1. Add **1 mL 1× RBC Lysis Buffer** (e.g. HybriMax) per lung.

2. Mix gently and incubate for **3–5 minutes at room temperature**.
3. Immediately top up to **50 mL** with cold FACS/MACS Buffer.
4. Centrifuge at **400 g for 5 minutes at 4 °C**.
5. Discard supernatant.

> **Note**
>
> If the lung was perfused effectively, this step may not be needed.

STEP 6 – Final wash and resuspension

1. Loosen pellet by gentle flicking or brief vortexing.
2. Resuspend in **5 mL FACS/MACS Buffer** (or other desired volume).
3. Filter again through a **fresh 70 m strainer** if clumping is observed.
4. Keep the suspension **on ice** until staining, sorting, or single-cell workflows.

Buffers Required

Full composition, preparation, storage, and safety information for each buffer lives in its own linked buffer document — only a concise per-buffer summary is reproduced here, generated automatically from that document.

FACS Buffer

Component	Final concentration / amount
PBS 1x	to 500 mL
FCS	10 mL (2% v/v)

- **Final volume:** 500 mL
- **Preparation note:** Mix gently by inversion.
- **Sterilisation / filtering:** 0.22 um filter if sterility required
- **Storage:** 4C, 4-6 weeks
- **Caveat:** No EDTA - EDTA is excluded from FACS buffer to avoid interference with staining reagents and functional assays. Use MACS Buffer (BUF-MACS-BUF) instead if EDTA is needed.

Full composition, preparation steps, storage, and safety information: `BUF-FACS-BUF`.

MACS Buffer

Component	Final concentration / amount
PBS 1x	to 500 mL
FCS	10 mL (2% v/v)
EDTA (0.5 M)	2 mL (2 mM final)

- **Final volume:** 500 mL
- **Preparation note:** Mix gently by inversion.
- **Sterilisation / filtering:** 0.22 um filter if sterility required
- **Storage:** 4C, 4-6 weeks
- **Caveat:** Contains EDTA - do not use for liver perfusion or any step upstream of collagenase digestion (EDTA chelates the Ca2+/Zn2+ cofactors collagenases require). Use FACS Buffer (BUF-FACS-BUF) instead.

Full composition, preparation steps, storage, and safety information: `BUF-MACS-BUF`.

RBC Lysis Buffer (HybriMax)

Component	Final concentration / amount
RBC Lysis Buffer (HybriMax), 1x ready-to-use	as supplied

- **Final volume:** 0.5 mL per sample (as used in `MUS-LIV-DIG`)

- **Preparation note:** If supplied as concentrate, dilute to 1x per manufacturer instructions before use.
- **Storage:** 2-8C per manufacturer instructions; do not freeze unless explicitly supported
- **Caveat:** Used at 0.5 mL of 1x buffer per sample with a 7-minute room-temperature incubation in `MUS-LIV-DIG` - adapt incubation time if switching supplier.

Full composition, preparation steps, storage, and safety information: `BUF-RBC-HYB`.

ACK RBC Lysis Buffer

Component	Final concentration / amount
Ammonium chloride (NH4Cl)	8.26 g/L
Potassium bicarbonate (KHCO3)	1.00 g/L
EDTA	0.037 g/L (37 mg/L)

- **Final volume:** 1 L
- **Preparation note:** Dissolve in sterile distilled water, then adjust to pH 7.2 with dilute HCl before sterile filtration.
- **Sterilisation / filtering:** 0.22 um filter required
- **Storage:** 4C; stable up to 3 months when sterile; do not freeze.

Full composition, preparation steps, storage, and safety information: `BUF-ACK-LYSIS`.

Materials

Reagents

Reagent	Supplier / Cat#	Notes
Lung Digestion Buffer	In-house	Pre-warmed to 37 °C
FACS/MACS Buffer	In-house	`BUF-FACS-BUF` / `BUF-MACS-BUF`; cold
RBC Lysis Buffer (1×)	[TBD]	Use only if needed
PBS (1×)	Any	For perfusion and rinsing

Disposables

Item	Notes
50 mL Falcon tubes	1 per lung
10 cm petri dishes	For mincing & filtering
70 m strainers	Do not reuse
5 mL syringes (no needle)	For mechanical dissociation
Scissors, scalpels	For mincing
Transfer pipettes	For washing & transfers

Equipment

Equipment	Notes
Benchtop shaker/incubator	37 °C, 250 rpm
Benchtop centrifuge	400 g at 4 °C
Biosafety cabinet (Class II)	Recommended
Timer	For digestion and lysis steps

Troubleshooting

Issue	Possible cause	Suggested solution
Persistent clumps	Incomplete digestion	Increase mechanical dissociation; do not over-digest
High RBC contamination	Lungs not perfused	Perform RBC lysis or perfuse before collection
Low viability	Over-digestion or RT handling	Limit digestion to 30–35 min; keep washes cold
Excess debris	Harsh mechanical disruption	Use gentle, repetitive plunger strokes

Safety (brief)

- Treat mouse tissues as potentially infectious and process according to institutional S1/S2 rules.
- Enzyme mixtures (Collagenase, DNase) may cause irritation; wear gloves and eye protection.
- Dispose of sharps, tissues, and enzymatic waste according to facility guidelines.

Version history

Version	Date	Author	Change summary
v1.0	2025-11-20	Dillon Corvino	Initial upgraded version for lung digestion
v1.1	2026-09-18	Dillon Corvino	Replaced `# Buffers used` (bare `library(tidyverse)`, leaked R package-startup warnings into the rendered document) with an auto-generated `# Buffers Required` section, kept in its correct position before Materials; Materials table FACS/MACS Buffer row now lists both `BUF-FACS-BUF` and `BUF-MACS-BUF` (registry split into separate EDTA-free/EDTA-containing buffers; prose wording left as "FACS/MACS Buffer" since the distinction does not matter to this procedure).