Mouse Spleen Dissociation to Single-Cell Suspension

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Purpose

This protocol describes the rapid isolation of leukocytes from mouse spleen using mechanical dissociation, red blood cell lysis, and gentle washing steps. The resulting suspension is suitable for flow cytometry, sorting, and standard immunological assays.

Critical notes (read before starting)

- Work on **ice** or at **4 °C** after dissociation to preserve viability.
- **Do not reuse strainers** between spleens.
- RBC lysis should be **time-limited (5 minutes)** to prevent leukocyte damage.

Approximate timing

- Dissection and spleen removal: **2–3 min**
- Mechanical dissociation: **2–3 min**
- RBC lysis: **5 min**
- Final wash and resuspension: **5 min**

Total hands-on time per spleen: **15–20 minutes**.

Table of contents

Purpose 1

Critical notes (read before starting) 1

Approximate timing 1

Procedure 3
- STEP 1 – Collection and preparation . . . 3
- STEP 2 – Mechanical dissociation . . . 3
- STEP 3 – Wash and centrifugation . . . 3
- STEP 4 – RBC lysis . . . 3
- STEP 5 – Filtration and final wash . . . 4
- STEP 6 – Downstream handling . . . 4

Buffers Required 5
- FACS Buffer . . . 5
- RBC Lysis Buffer (HybriMax) . . . 5
- ACK RBC Lysis Buffer . . . 5

Materials 7
- Reagents . . . 7
- Disposables . . . 7
- Equipment . . . 7

Troubleshooting 8

Safety (brief) 9

Version history 9

Procedure

STEP 1 – Collection and preparation

1. Euthanise the mouse by an approved method.

2. Expose the spleen by opening the abdominal cavity.

3. Excise the spleen and remove connective tissue & adipose tissue
4. Place it into a petri dish or 15 mL Falcon tube containing **cold PBS or FACS Buffer**.

STEP 2 – Mechanical dissociation

1. Place a **70 µm cell strainer** over a **50 mL Falcon tube**.

2. Transfer the spleen onto the strainer.

3. Using the **back of a 5 mL syringe plunger**, gently mash the spleen through the mesh until a homogenous suspension is obtained.

4. Rinse the strainer with **FACS Buffer** and top the tube up to **50 mL**.

> **Note**
>
> Max of 2 spleens per tube/strainer

STEP 3 – Wash and centrifugation

1. Centrifuge the 50 mL tube at **400 g for 5 minutes at 4 °C**.

2. Carefully pour off the supernatant.

STEP 4 – RBC lysis

1. Add **RBC lysis buffer** to the cell pellet (**1 mL per spleen**):
   - **ACK lysis buffer:** incubate for **1.5–2 minutes at room temperature**, **or**
   - **HybriMax RBC Lysis Buffer:** incubate for **4–5 minutes at room temperature**.

2. Gently resuspend immediately by pipetting or flicking to ensure uniform lysis.

3. Quench the reaction by topping up to **50 mL with FACS Buffer**.
4. Centrifuge at **400 g for 5 minutes at 4 °C**.

> **Warning**
>
> Do not exceed the indicated incubation times. Over-incubation is the most common cause of reduced leukocyte viability. If red cells remain, repeat a **short second lysis** rather than extending the incubation.

> **Note**
>
> ACK lysis buffer is **stronger and faster** but less forgiving. HybriMax is **milder and preferred** when viability is critical (e.g. downstream scRNA-seq or adoptive transfer).

STEP 5 – Filtration and final wash

1. Filter the suspension through a **fresh 70 µm strainer** into a clean tube.
2. Centrifuge at **400 g for 5 minutes at 4 °C**.
3. Carefully discard supernatant.
4. Resuspend the pellet in **5 mL FACS Buffer** (or a volume appropriate for your downstream assay).

STEP 6 – Downstream handling

Proceed immediately to:

- Flow cytometry staining
- FACS sorting
- Cryopreservation
- Functional assays

Keep cells **on ice** until use.

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`.

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
PBS or FACS Buffer	In-house	`BUF-FACS-BUF`; cold
HybriMax RBC Lysis Buffer	Sigma	1 mL per spleen
Sterile PBS	Any	For handling and rinsing

Disposables

Item	Notes
50 mL Falcon tubes	1 per spleen
70 µm cell strainers	Do not reuse
5 mL syringes (without needle)	For mashing
Transfer pipettes	For washing/rinsing

Equipment

Equipment	Notes
Biosafety cabinet	Recommended
Benchtop centrifuge	400 g at 4 °C
Petri dishes	For dissection/dissociation
Timer	For RBC lysis

Troubleshooting

Issue	Possible cause	Suggested solution
Low cell yield	Incomplete mechanical dissociation	Spend additional time mashing through mesh
Excess debris	Over-aggressive mashing	Apply gentler pressure
Poor viability	Prolonged room-temperature handling	Keep samples cold; minimise processing time
Pellets lost	Pouring too forcefully	Use slow, controlled decanting

Safety (brief)

- Process mouse tissues according to S1/S2 guidelines and ethics approval.
- RBC lysis buffer contains toxic reagents — wear PPE and avoid inhalation or skin contact.
- Dispose of biological material and sharps appropriately.

Version history

Version	Date	Author	Change summary
v1.0	2025-11-20	Dillon Corvino	Initial upgraded version for spleen
v1.1	2025-11-26	Dillon Corvino	Manual check and update protocol
v1.2	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; added `BUF-FACS-BUF` ID to the FACS Buffer reagent row.