

# Mouse Spleen Dissociation to Single-Cell Suspension

Abdullah Lab, IMMEI, University Hospital Bonn

2025-11-26

**Protocol ID:** MUS-SPL-DIS-001

**Version:** v1.1

**Author:** Dillon Corvino

## 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**.

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## 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**.

#### **i** 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 used

- FACS Buffer – BUF-FACS-v1.0
- RBC Lysis Buffer (HybriMax) – BUF-RBC-HYB-v1.0

## Materials

### Reagents

Reagent	Supplier / Cat#	Notes
PBS or FACS Buffer	In-house	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 $\mu$ m 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