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.
- Spleen pellets can be **fragile** after lysis — pour supernatants slowly.

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: **10–15 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 place it into a petri dish containing **cold PBS or FACS/MACS Buffer**.

STEP 2 – Mechanical dissociation

1. Place a **70 m 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/MACS Buffer** and top the tube up to **50 mL**.

> Tip
>
> Use gentle, consistent pressure — avoid grinding, which reduces viability.

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 **1 mL HybriMax RBC Lysis Buffer** (or equivalent) **per spleen** to the pellet.
2. Gently resuspend the pellet by pipetting or flicking.
3. Incubate for **5 minutes at room temperature**.
4. Top up to **50 mL** with **FACS/MACS Buffer**.

> Warning
>
> Over-lysis (>5 minutes) can reduce leukocyte viability and alter scatter profiles.

STEP 5 – Filtration and final wash

1. Filter the suspension through a **fresh 70 m 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/MACS 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/MACS Buffer** – `BUF-FACS-v1.0`
- **RBC Lysis Buffer (HybriMax)** – `BUF-RBC-HYB-v1.0`

Materials

Reagents

Reagent	Supplier / Cat#	Notes
PBS or FACS/MACS 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 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