

Mouse Blood Processing to Single-Cell Suspension

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Purpose

This protocol describes the processing of mouse peripheral blood collected in EDTA to generate a leukocyte-enriched single-cell suspension using RBC lysis and gentle washing. The final suspension is suitable for flow cytometry, basic immunophenotyping, and other standard immunological assays.

Critical notes (read before starting)

- Always collect blood into tubes containing **EDTA** (or other approved anticoagulant) and **mix immediately** to prevent clotting.
- Perform all processing steps as soon as possible after collection; avoid prolonged storage at room temperature.
- RBC lysis should be **time-limited (~5 minutes)** to minimise leukocyte damage.
- Keep samples on **ice** or at **4 °C** after lysis and during washing to preserve viability.

Approximate timing

- Blood collection and transfer: **2–5 min per mouse**
- Initial spin and serum removal: **5 min**
- RBC lysis and wash: **10–15 min**

Total hands-on time per sample: **20–25 minutes**, depending on batching.

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Procedure

STEP 1 – Blood collection

1. Prepare **EDTA-containing microcentrifuge tubes** (20–40 μ L EDTA per tube) and label one tube per mouse.
2. Collect blood using an **insulin syringe** (or other approved method, e.g. retro-orbital, submandibular, cardiac puncture as per ethics approval).
3. Immediately dispense the blood into the EDTA tube.
4. Flick the tube gently to mix blood with EDTA and prevent clotting.

Warning

Ensure local ethics approval covers the chosen blood collection method. Handle sharps carefully and discard immediately into an approved sharps container.

STEP 2 – Allocate sample volume and initial spin

1. Transfer a defined volume of well-mixed whole blood (e.g. **100–200 μ L**, or as required) from the EDTA tube into a **new, labelled microcentrifuge tube**.
 - Record the volume used per sample in your lab notebook.
2. Centrifuge the whole blood at **maximum g (bench-top microcentrifuge)** for **5 minutes** at room temperature or 4 $^{\circ}$ C.
3. Carefully remove the **serum**:
 - Discard, or
 - Aliquot and freeze at **–20 $^{\circ}$ C** or **–80 $^{\circ}$ C** if required for serum analyses.

STEP 3 – RBC lysis

1. Gently resuspend the remaining blood pellet in **1 mL of 1 \times RBC lysis buffer** (e.g. BioLegend / HybriMax 1 \times).
2. Transfer the suspension to a **15 mL Falcon tube**.
3. Gently mix by inversion or brief pipetting to ensure complete resuspension.
4. Incubate for **5 minutes at room temperature**.

Warning

Do not exceed the recommended lysis time; prolonged exposure to RBC lysis buffer can damage leukocytes and alter marker expression.

STEP 4 – Wash and final resuspension

1. After the 5-minute lysis, top up the tube to **10 mL** with **FACS/MACS Buffer**.
2. Centrifuge at **400 g for 4–5 minutes at 4 °C**.
3. Carefully pour off or aspirate the supernatant.
4. If substantial red cell contamination remains:
 - Repeat the RBC lysis step (STEP 3) once more, followed by the wash in STEP 4.
5. Finally, resuspend the cell pellet in an appropriate volume of **FACS/MACS Buffer** (e.g. **0.5–1 mL**) for:
 - Flow cytometry staining
 - Cell counting
 - Other downstream assays

Keep the cell suspension on **ice** until staining.

Buffers used

- FACS/MACS Buffer – BUF-FACS-v1.0
- RBC Lysis Buffer (e.g. BioLegend / HybriMax) – BUF-RBC-HYB-v1.0

Materials

Reagents

Reagent	Supplier / Cat#	Notes
EDTA	[TBD]	20–40 L per collection tube
RBC Lysis Buffer (1×)	[TBD]	1 mL per sample; may repeat once
FACS/MACS Buffer	In-house (see buffer)	For washing and resuspension
PBS 1×	Any	As needed

Disposables

Item	Notes
Microcentrifuge tubes (1.5–2 mL)	2 per mouse (EDTA collection + processing)
15 mL Falcon tubes	1 per sample
Insulin syringes / collection needles	1 per mouse (as per procedure)
Transfer pipettes	For serum removal and transfers
Gloves, bench wipes, sharps container	Standard PPE and safety disposables

Equipment

Equipment	Notes
Bench-top microcentrifuge	For initial high-g spin
Centrifuge for 15 mL tubes	400 g at 4 °C
Biosafety cabinet (Class II)	Recommended for handling blood
Timer	For lysis timing

Troubleshooting

Issue	Possible cause	Suggested solution
Sample clotted	Delayed mixing with EDTA	Mix immediately after blood collection; discard clotted samples
Persistent red tinge after lysis	Incomplete RBC lysis	Repeat lysis once; do not extend lysis time excessively
Low leukocyte recovery	Pellet loss during washes	Pour supernatants slowly; consider aspirating instead
Poor viability	Prolonged room-temperature handling	Keep samples cold; minimise processing time

Safety (brief)

- Treat all mouse blood as potentially infectious; follow S1/S2 biosafety rules.
- Use appropriate PPE (lab coat, gloves, eye protection) and handle sharps with care.
- RBC lysis buffers may contain toxic components (e.g. ammonium chloride, azide); avoid contact with skin and eyes and dispose of waste according to institutional chemical safety guidelines.

Version history

Version	Date	Author	Change summary
v1.0	2025-11-20	Dillon Corvino	Initial upgraded version for blood processing