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 µL 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 Serum removal

1. Transfer a defined volume of well-mixed whole blood (e.g. **100–200 µL**, 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 °C.
3. Carefully remove the **serum**:
    - Discard, or
    - Aliquot and freeze at **−20 °C or −80 °C** if required for serum analyses.

STEP 3 – RBC lysis

1. Gently resuspend the remaining blood pellet in **1 mL of 1× BioLegend RBC lysis buffer**.
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 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 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 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
EDTA	[TBD]	20–40 µL per collection tube
BioLegend RBC Lysis Buffer (1×)	[TBD]	1 mL per sample; may repeat once
FACS Buffer	In-house (see buffer)	`BUF-FACS-BUF`; 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
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.