

# 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.

## Table of contents

<b>Purpose</b>	<b>1</b>
<b>Critical notes (read before starting)</b>	<b>1</b>
<b>Approximate timing</b>	<b>1</b>
<b>Procedure</b>	<b>3</b>
STEP 1 – Blood collection . . . . .	3
STEP 2 – Allocate sample volume and initial spin . . . . .	3
STEP 3 – RBC lysis . . . . .	3
STEP 4 – Wash and final resuspension . . . . .	4
<b>Buffers used</b>	<b>5</b>
<b>Materials</b>	<b>6</b>
Reagents . . . . .	6
Disposables . . . . .	6
Equipment . . . . .	6
<b>Troubleshooting</b>	<b>7</b>
<b>Safety (brief)</b>	<b>8</b>
<b>Version history</b>	<b>8</b>

## Procedure

### STEP 1 – Blood collection

1. Prepare **EDTA-containing microcentrifuge tubes** (20–40  $\mu$ 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  $\mu$ 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