Mouse Bone Marrow Isolation to Single-Cell Suspension

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Purpose

This protocol describes the isolation of bone marrow cells from mouse long bones (typically femurs and tibias) by flushing with buffer, mechanical dispersion, and optional RBC lysis. The resulting suspension is suitable for flow cytometry, FACS sorting, and functional assays.

Critical notes (read before starting)

- Work on **ice** or at **4 °C** after marrow isolation to preserve viability.
- Keep bones **moist at all times** in cold buffer; do not allow them to dry.
- Use fresh, sterile instruments and avoid introducing bone shards into the final suspension.
- If erythrocyte content is high, perform **controlled RBC lysis**; avoid prolonged exposure to lysis buffer.

Approximate timing

- Bone dissection: **10–15 min per mouse** (faster when batching)
- Marrow flushing and dispersion: **10–15 min**
- RBC lysis and washing: **10–15 min**

Total hands-on time per mouse: **30–45 minutes**, depending on experience and batching.

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Procedure

STEP 1 – Bone collection

1. Euthanise the mouse using an approved method.
2. Spray the hind limbs with **70% ethanol** and transfer the carcass to a sterile working area (e.g. Class II biosafety cabinet, if available).
3. Dissect out **femurs** using scissors and forceps.
4. Remove all excess muscle and connective tissue from the bones.
5. Place cleaned bones in a 50 mL tube or petri dish containing **cold FACS Buffer or PBS** and keep on ice.

> Tip
>
> Note how many bones per mice for later cell number calculations

STEP 2 – Bone marrow extraction

Method 1 - Flushing the bone marrow

1. In a petri dish or on a sterile surface, cut off the **epiphyses** (ends) of each bone with sterile scissors to expose the marrow cavity.
2. Fill a **10 mL syringe** fitted with an appropriate gauge needle (e.g. 25 G) with **cold FACS Buffer or PBS**.
3. Position the needle into one end of the bone and flush the marrow into a **petri dish** or **50 mL tube**:
   - Use ~1–3 mL buffer per bone, depending on size.
4. Repeat flushing from both ends if necessary until the bone appears pale and empty.
5. Gently triturate the flushed marrow with a **5–10 mL pipette** or by drawing up and down with the syringe (without needle) to break up clumps.

> Warning
>
> Avoid vigorous pipetting that shears cells; use gentle, repeated strokes to obtain a single-cell suspension.

Method 2 - Centrifugation

1. Poke a small hole (using a needle) in a PCR tube
2. Place PCR tube in an Eppendorf tube
3. Add 200 µL cold **FACS Buffer**
4. Cut off the **epiphyses** (ends) of each bone with sterile scissors to expose the marrow cavity.
5. Spin in table-top micro-centrifuge for 30 sec - 2 mins
6. Ensure bone marrow has not gotten stuck in the PCR tube and bones appear pale and empty (Repeat spin as needed)

STEP 3 – Filtration and initial wash

1. Pass sample through a **70 µm cell strainer** onto a **15 mL Falcon tube**.
2. Rinse the petri dish/source tube and strainer with additional cold **FACS Buffer** to collect remaining cells and top up to **15 mL**.
3. Centrifuge at **400 g for 5 minutes at 4 °C**.
4. Carefully discard the supernatant.

STEP 4 – RBC lysis (if required)

1. Gently resuspend the bone marrow pellet in **100 µL 1× RBC Lysis Buffer** (e.g. HybriMax or equivalent).
2. Incubate for **5 minutes at room temperature**, gently inverting the tube once or twice.

> ⚠ Warning
>
> Do not exceed 5 minutes of RBC lysis; prolonged exposure can reduce leukocyte viability and alter surface marker expression.

3. After lysis, immediately top up the tube to **10 mL** with **FACS Buffer**.
4. Centrifuge at **400 g for 5 minutes at 4 °C**.
5. Carefully discard the supernatant.

STEP 5 – Final wash and resuspension

1. Resuspend cells in **1 mL FACS Buffer** (adjust volume according to desired final concentration and downstream application).
2. Gently flick or briefly vortex to loosen the pellet.
3. If residual clumping is observed, pass the suspension again through a **70 µm strainer** into a fresh tube.
4. Keep the suspension on **ice** until counting, staining, or further processing.

STEP 6 – Downstream applications

Proceed to:

- Cell counting and viability assessment.
- Flow cytometry staining panels (e.g. hematopoietic profiling).
- FACS sorting (e.g. HSCs, progenitors, mature lineages).
- Functional assays or in vitro culture as per experimental design.

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
FACS Buffer	In-house (see buffer)	`BUF-FACS-BUF`; cold; used for flushing and washes
PBS 1× (sterile)	Any	Optional; for flushing
RBC Lysis Buffer (1×)	[TBD]	1–2 mL per pellet; as needed
70% Ethanol	Any	For surface disinfection of carcass

Disposables

Item	Notes
50 mL Falcon tubes	For bone storage and processing
15 mL Falcon tubes	Optional; for smaller volumes
70 µm cell strainers	For debris removal
10 mL syringes + needles (~25 G)	For marrow flushing
5–10 mL serological pipettes	For trituration
Petri dishes	For bone trimming and flushing
Gloves, bench wipes	Standard PPE and cleanup

Equipment

Equipment	Notes
Biosafety cabinet (Class II)	Recommended for tissue handling
Benchtop centrifuge	400 g at 4 °C, compatible with 50 mL tubes
Dissection scissors and forceps	For bone isolation and cleaning
Timer	For RBC lysis timing

Troubleshooting

Issue	Possible cause	Suggested solution
Low cell yield	Incomplete marrow flushing	Flush from both ends; increase volume per bone slightly
Excess debris or bone fragments	Inadequate filtration	Ensure proper use of 70 µm strainer; repeat filtration if needed
High red cell contamination	Insufficient RBC lysis	Repeat RBC lysis briefly (3 min) followed by wash
Poor viability	Overly vigorous trituration or long RT handling	Use gentler pipetting; keep steps cold; minimise processing time

Safety (brief)

- Handle mouse tissues and sharps according to institutional S1/S2 and animal ethics guidelines.
- RBC lysis buffers may contain hazardous components; avoid skin/eye contact and dispose of waste according to chemical safety rules.
- Bone fragments and needles must be discarded into appropriate sharps containers.

Version history

Version	Date	Author	Change summary
v1.0	2025-11-20	Dillon Corvino	Initial upgraded version for bone marrow isolation
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.