Mouse Tumour Digestion to Single-Cell Suspension

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2026-09-18

Protocol ID: MUS-TMR-DIG
Version: v1.1
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Purpose

This protocol describes the processing of mouse solid tumours to generate high-quality single-cell suspensions using enzymatic digestion (Collagenase + DNase), thorough mechanical dissociation, and controlled washing. The resulting suspensions are suitable for flow cytometry, FACS sorting, tumour-infiltrating lymphocyte (TIL) profiling, and single-cell omics workflows.

Critical notes (read before starting)

- **Tumours vary widely** in stiffness and extracellular matrix composition; adjust mechanical dissociation effort accordingly.
- For large or fibrotic tumours, digestion efficiency increases when the tissue is minced **finely** before enzyme incubation.
- Use **fresh digestion buffer** with DNase added on the day of use.
- Do not over-digest: aim for **30–45 minutes**, depending on tumour type.
- After digestion, keep samples on **ice** to maximise viability.
- Avoid introducing **hair, necrotic debris, or capsule tissue**, which reduce filter performance and cell quality.

Approximate timing

- Tumour excision + trimming: **5–10 min**
- Mincing: **5 min**
- Enzymatic digestion: **30–45 min**
- Filtering + washes: **20–30 min**

Total hands-on time: ~**60–90 minutes**, depending on tumour type and size.

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Procedure

STEP 1 – Tumour excision and preparation

1. Euthanise the mouse using an approved method.
2. Expose the tumour and excise it with sterile scissors and forceps.
3. Remove fat, necrotic outer layers, and connective tissue using dissection scissors.
4. Rinse tumour briefly in **cold PBS** to remove blood and debris.
5. Transfer to a **10 cm petri dish** containing cold **PBS or FACS/MACS Buffer**.

> Tip
>
> Remove necrotic regions; they clog strainers and reduce cell viability.

STEP 2 – Mincing

1. On a sterile petri dish, mince tumour tissue into **very small pieces** using scalpels or scissors.
   - Aim for **1–2 mm** fragments for optimal enzyme penetration.
2. Transfer minced tissue into a **50 mL Falcon tube** containing **5 mL Tumour Digestion Buffer** (`BUF-TMR-DIG-v1.0`).

STEP 3 – Enzymatic digestion

1. Place tubes on a **37 °C benchtop shaker** at **250 rpm**.
2. Incubate for **30–45 minutes**, depending on tumour density.
3. Every 10–15 minutes, remove the tube and gently shake or invert to encourage even digestion.

> Warning
>
> Over-digestion (>45 min) can reduce viability, especially in lymphocytes and myeloid cells.

STEP 4 – Mechanical dissociation

1. Add **FACS/MACS Buffer** to bring total volume to **20–25 mL**.
2. Pour the digested tumour onto a **70 m strainer** placed over a **10 cm petri dish**.
3. Using the **back of a 5 mL syringe plunger**, mash the tissue thoroughly through the strainer.

4. Rinse the strainer with additional cold **FACS/MACS Buffer** until no visible tissue remains.
5. Transfer the filtrate back into a clean **50 mL tube**.
6. Top up to **50 mL** with cold FACS/MACS Buffer.
7. Centrifuge at **400 g for 5 minutes at 4 °C**.
8. Carefully discard the supernatant.

STEP 5 – RBC lysis (if required)

1. If the suspension appears red or contains substantial blood, add **1 mL 1× RBC Lysis Buffer** (HybriMax or equivalent).
2. Mix gently and incubate for **3–5 minutes at room temperature**.
3. Immediately top up to **50 mL** with cold FACS/MACS Buffer.
4. Centrifuge at **400 g for 5 minutes at 4 °C**.
5. Discard supernatant.

STEP 6 – Final wash and filtration

1. Loosen the pellet by gentle flicking.
2. Resuspend in **5–10 mL FACS/MACS Buffer**.
3. Filter again through a **fresh 70 m strainer** to remove remaining debris and aggregates.
4. Keep the final cell suspension **on ice** for downstream applications.

STEP 7 – Downstream applications

Proceed to:

- Flow cytometry / TIL profiling
- FACS sorting
- Tumour microenvironment analyses
- Cryopreservation of tumour-derived cell subsets
- Single-cell RNA-seq / TCR-seq workflows

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`.

MACS Buffer

Component	Final concentration / amount
PBS 1x	to 500 mL
FCS	10 mL (2% v/v)
EDTA (0.5 M)	2 mL (2 mM final)

- **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:** Contains EDTA - do not use for liver perfusion or any step upstream of collagenase digestion (EDTA chelates the Ca2+/Zn2+ cofactors collagenases require). Use FACS Buffer (BUF-FACS-BUF) instead.

Full composition, preparation steps, storage, and safety information: `BUF-MACS-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
Tumour Digestion Buffer	In-house	Pre-warmed to 37 °C
FACS/MACS Buffer	In-house	`BUF-FACS-BUF` / `BUF-MACS-BUF`; cold
RBC Lysis Buffer	[TBD]	Optional; 1 mL per tumour
PBS 1×	Any	For rinsing

Disposables

Item	Notes
50 mL Falcon tubes	1 per tumour
10 cm petri dishes	For mincing and filtering
70 m strainers	Do not reuse
5 mL syringes (no needle)	For mechanical dissociation
Scalpel blades / scissors	For fine mincing
Transfer pipettes	For washes

Equipment

Equipment	Notes
Benchtop shaker/incubator	37 °C, 250 rpm
Benchtop centrifuge	400 g at 4 °C
Biosafety cabinet (Class II)	Recommended
Timer	For digestion and lysis

Troubleshooting

Issue	Possible cause	Suggested solution
Large undigested chunks	Insufficient mincing or digestion time	Mince more finely; extend digestion by 5–10 min
Excess debris	Too much necrotic/capsular tissue	Trim tumour more aggressively before mincing
High RBC content	No perfusion / vascular tumour	Perform RBC lysis once
Poor viability	Over-digestion or harsh mashing	Reduce digestion time; use gentler plunger pressure

Safety (brief)

- Treat tumours as potentially infectious and follow S1/S2 safety requirements.
- Collagenase and DNase can irritate skin and eyes—wear full PPE.
- Dispose of tumour tissue, blades, and enzymatic waste in biohazard and sharps containers as applicable.

Version history

Version	Date	Author	Change summary
v1.0	2025-11-20	Dillon Corvino	Initial upgraded version for tumour digestion
v1.1	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; Materials table FACS/MACS Buffer row now lists both `BUF-FACS-BUF` and `BUF-MACS-BUF` (registry split into separate EDTA-free/EDTA-containing buffers; prose wording left as "FACS/MACS Buffer" since the distinction does not matter to this procedure).