Mouse Liver Digestion Buffer with Collagenase D

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Protocol ID: BUF-MUS-LIV-DIG-COL-D-001
Version: v1.1
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Purpose

This buffer is used for enzymatic digestion of mouse liver tissue prior to preparation of single-cell suspensions. It supports efficient dissociation while aiming to preserve cell viability for downstream flow cytometry, FACS sorting, and single-cell omics workflows.

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Linked protocols

This buffer is used in:

- **Mouse Liver Dissociation to Single-Cell Suspension** – `LIV-001` (v1.0)

Composition (final working buffer)

Final working-buffer composition per **5 mL**:

Component	Stock concentration	Volume for 5 mL	Final concentration (approx.)	Notes
DMEM (incomplete)	—	to 5 mL	N/A	Base medium
Collagenase D	100 mg/mL	10 µL	1 mg/mL	Reconstituted from powder stock
DNase I	10 mg/mL	1 µL	10 µg/mL	Added on day of use

> **Note**
>
> The table above describes the **final working buffer** used in the protocol. In practice, a **stock digestion buffer without DNase** is prepared and frozen in aliquots, and DNase is added only on the day of use (see Preparation and Storage sections).

Reconstitution of Collagenase D powder (stock preparation)

Collagenase D is supplied as a **lyophilised powder** and must be reconstituted to generate a concentrated stock solution for preparation of liver digestion buffer.

1. Perform all steps **under a biosafety cabinet** using sterile technique.
2. Add **1 mL sterile PBS (1×)** directly to a vial containing **100 mg Collagenase D powder**.
3. Recap the vial securely and **invert gently several times** until all powder is fully dissolved.
    - Avoid vigorous shaking or vortexing.
    - Inspect the vial to ensure no undissolved powder remains on the walls or cap.
4. This yields a **100 mg/mL Collagenase D stock solution**.
5. Aliquot the stock into **sterile 1.5 mL microcentrifuge tubes**, typically **100 µL per tube**.
6. Label aliquots with:
    - "Collagenase D 100 mg/mL"
    - Date of preparation
    - Initials

7. Freeze aliquots at **−20 °C** for long-term storage.

> ⚠ Warning
>
> Collagenase powder is a **respiratory and skin irritant**.
> Handle the dry powder carefully, avoid aerosol generation, and always work under a biosafety cabinet while wearing appropriate PPE (lab coat, gloves, and eye protection).

> ℹ Note
>
> Aliquoting into single-use volumes avoids repeated freeze–thaw cycles, which can reduce enzymatic activity and introduce variability between experiments.

Preparation

A. Prepare "Liver Digestion Buffer (no DNase)"

This buffer is DMEM + Collagenase D, **without DNase**. It is frozen in aliquots for convenient use.

> ℹ Note
>
> Collagenase and DNase I are **proteins and mechanically sensitive**. Avoid vigorous vortexing or shaking, as this can denature the enzymes and reduce activity. Mix by gentle inversion or slow pipetting to preserve enzymatic function.

1. Thaw a **Collagenase D 100 mg/mL stock aliquot** on ice or at 4 °C.
2. In a sterile tube, prepare the digestion stock for the desired number of aliquots, for example:
   - For each **5 mL** stock aliquot:
     - Add **5 mL** DMEM (incomplete).
     - Add **10 µL** Collagenase D stock (100 mg/mL).
     - Mix gently by inversion (avoid foaming).
3. Aliquot the digestion buffer into sterile tubes (e.g. **5–10 mL per aliquot**, depending on typical experimental usage, note: 5 mL per liver).
4. Label aliquots clearly with:
   - "Liver Digestion Buffer (no DNase)"
   - Date
   - Initials
5. Freeze aliquots at **−20 °C**.

B. Prepare final working Liver Digestion Buffer (day of use)

1. Remove the required number of **Liver Digestion Buffer (no DNase)** aliquots from −20 °C and **thaw** at room temperature or 4 °C.
2. For each **5 mL** of final working buffer needed:
    1. Ensure the thawed buffer is well mixed (gentle inversion).
    2. Add **1 µL DNase I stock (10 mg/mL)** to **5 mL** digestion buffer
    3. Mix gently by inversion.
3. Pre-warm the final working buffer to **37 °C** before use (e.g. water bath or incubator).
4. If desired, pre-aliquot **5 mL** of the final working buffer into pre-labelled 50 mL tubes for liver digestion.

> **Tip**
>
> To minimise variability, prepare all working-buffer aliquots for a given experiment at the **same time** using the **same DNase stock**, and keep buffers at 37 °C only for as long as required during digestion.

> **Note**
>
> DNase I is added **only on the day of use** because prolonged storage in digestion buffer containing active collagenase can lead to **proteolytic degradation of DNase**.

Storage and stability

Liver Digestion Stock (no DNase)

- **Composition:** DMEM (incomplete) + Collagenase D (1 mg/mL final when diluted as described).
- **Storage:** Store aliquots at **−20 °C**.
- **Stability:** Use within **3–6 months** of preparation, or according to internal lab validation.
- **Handling:** Avoid repeated freeze–thaw cycles; thaw gently and mix before use.

Final working Liver Digestion Buffer (with DNase)

- Prepared from thawed **Liver Digestion Stock (no DNase)** + DNase I.
- **Use on the same day** as preparation.
- Keep at **37 °C** only during active digestion; otherwise keep at room temperature briefly.
- Do not refreeze once DNase has been added; discard unused buffer at the end of the day.

Stock reagents

- **Collagenase D stock (100 mg/mL)**
  - Store at **−20 °C** in aliquots.
  - Avoid repeated freeze–thaw cycles.
- **DNase I stock (10 mg/mL)**
  - Store at **−20 °C** according to manufacturer's instructions.
  - Avoid repeated freeze–thaw cycles.
- **DMEM (incomplete)**
  - Store at **4 °C**, protected from light.
  - Respect manufacturer's expiry date.

Reagent details

Component	Supplier	Cat#	Notes
DMEM (incomplete)	TBD	TBD	High-glucose DMEM without supplements
Collagenase D	Roche	11088858001	Supplied as powder; reconstituted to 100 mg/mL in sterile PBS
DNase I	TBD	TBD	Prepare 10 mg/mL stock; avoid repeated thawing

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Safety (brief)

- Handle mouse tissues and digestion buffers in accordance with institutional biosafety rules (S1/S2 as applicable).

- Enzymes such as collagenase and DNase can cause irritation; wear appropriate PPE.
- Avoid inhalation of powders and contact with eyes or damaged skin.
- Dispose of digestion buffers and contaminated consumables as **biohazardous waste** according to local regulations.

Version history

Version	Date	Author	Change summary
v1.0	2025-11-20	Dillon Corvino	Initial buffer definition
v1.1	2025-12-29	Dillon Corvino	Added collagenase powder reconstitution and safety notes