Liver Digestion Buffer

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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	From frozen 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).

Preparation

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

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

1. Thaw **Collagenase D** stock (100 mg/mL) 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 ~**4 mL** DMEM (incomplete).
     - Add **10 µL** Collagenase D stock (100 mg/mL).
     - Mix gently by inversion (avoid foaming).
     - Bring volume to **5 mL** with DMEM (incomplete).
3. Aliquot the digestion stock into sterile tubes (e.g. **3–5 mL per aliquot**, depending on typical experimental usage).
4. Label aliquots clearly with:
   - "Liver Digestion Stock (no DNase)"
   - Concentration
   - Date
   - Version (`BUF-LIV-DIG-v1.0`)

5. Freeze aliquots at **−20 °C**.

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

1. Remove the required number of **Liver Digestion Stock (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 stock is well mixed (gentle inversion).
    2. Add **1 µL** of DNase I stock (10 mg/mL) to **5 mL** digestion stock.
    3. Mix gently by inversion.
3. Pre-warm the final working buffer to **37 °C** before use (e.g. by placing tubes in a 37 °C water bath or incubator).
4. If desired, pre-aliquot **3 mL** of the final working buffer into pre-labeled 50 mL liver collection tubes, and retain additional buffer in a separate tube for perfusion.

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

Storage and stability

Liver Digestion Stock (no DNase)

- **Composition**: DMEM (incomplete) + Collagenase D at 1 mg/mL final (when diluted as described).
- **Storage**:
    - Store aliquots at **−20 °C**.
    - Avoid repeated freeze–thaw cycles (use single-use aliquots where possible).
- **Stability**:
    - Recommended to use within **3–6 months** of preparation, or according to internal lab validation.
- Before use:
    - Thaw at room temperature or 4 °C.
    - Mix gently to ensure homogeneity.

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 steps; otherwise, keep at room temperature for short periods.
- Do not refreeze working buffer once DNase has been added; discard any unused working buffer at the end of the day.

Stock reagents

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

> **Note**
>
> If the lab validates alternative storage conditions (e.g. short-term storage of working buffer at 4 °C), document the conditions and update the version number and description accordingly.

Reagent details

Component	Supplier	Cat#	Notes
DMEM (incomplete)	[TBD]	[TBD]	High-glucose DMEM without supplements
Collagenase D	[TBD]	[TBD]	Prepare 100 mg/mL stock according to manufacturer
DNase I	[TBD]	[TBD]	Prepare 10 mg/mL stock; avoid repeated thawing

Safety (brief)

- Handle mouse tissues and enzymatic digestion buffers in accordance with institutional biosafety rules (S1/S2 as applicable).
- Collagenase and DNase are enzymes; avoid inhalation of powders and minimise contact with eyes or damaged skin.
- Dispose of buffer waste and contaminated consumables in appropriate **biohazard** or **chemical** waste streams, as determined by local regulations.

Version history

Version	Date	Author	Change summary
v1.0	2025-11-20	Dillon Corvino	Initial buffer definition