

# Liver Digestion Buffer

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**Protocol ID:** BUF-LIV-DIG-v1.0

**Version:** v1.0

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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  $-20\text{ }^{\circ}\text{C}$ .

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

1. Remove the required number of **Liver Digestion Stock (no DNase)** aliquots from  $-20\text{ }^{\circ}\text{C}$  and **thaw** at room temperature or  $4\text{ }^{\circ}\text{C}$ .
2. For each **5 mL** of final working buffer needed:
  1. Ensure the thawed stock is well mixed (gentle inversion).
  2. Add **1  $\mu\text{L}$**  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  **$37\text{ }^{\circ}\text{C}$**  before use (e.g. by placing tubes in a  $37\text{ }^{\circ}\text{C}$  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  $37\text{ }^{\circ}\text{C}$  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  $-20\text{ }^{\circ}\text{C}$ .
  - 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  $4\text{ }^{\circ}\text{C}$ .
  - 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  **$37\text{ }^{\circ}\text{C}$**  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  $-20\text{ }^{\circ}\text{C}$  in aliquots).
  - Avoid repeated freeze–thaw cycles.
- **DNase I stock (10 mg/mL)**
  - Store according to manufacturer’s instructions (often  $-20\text{ }^{\circ}\text{C}$ ).
  - Avoid repeated freeze–thaw cycles.
- **DMEM (incomplete)**
  - Store at  $4\text{ }^{\circ}\text{C}$ , 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  $4\text{ }^{\circ}\text{C}$ ), 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