

Mouse Liver Digestion Buffer with Collagenase IV

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Protocol ID: BUF-MUS-LIV-DIG-COL-IV-001

Version: v1.0

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Purpose

This buffer is used for enzymatic digestion of mouse liver tissue to generate viable single-cell suspensions. It contains Collagenase IV and DNase I at working concentrations suitable for dissociating liver parenchyma while preserving lymphocyte viability.

Working buffer composition

| Component | Final concentration | Notes |
|-------------------|---------------------|---------------------------------------|
| Collagenase IV | 0.5 mg/mL (example) | Adjust per validated stock; see below |
| DNase I | 25–50 µg/mL | Reduces clumping; protects viability |
| PBS (1×), sterile | — | Solvent |

Note

The final working concentrations here follow the proportions described in the original SOP (100 µL Collagenase IV into 10 mL PBS + 50 µL DNase I). Adjust exact activity units according to vendor.

Preparation

Stock solutions

| Reagent | Typical stock concentration | Storage |
|----------------|-----------------------------------|---------------------|
| Collagenase IV | 100 mg/mL in PBS or HBSS | Aliquoted at −20 °C |
| DNase I | 10 mg/mL in buffer (manufacturer) | Aliquoted at −20 °C |

Preparation of 10 mL working buffer (2×)

1. Thaw aliquots of Collagenase IV and DNase I on ice.
2. In a sterile tube, add:
 - **100 µL** Collagenase IV stock
 - **50 µL** DNase I stock
3. Add **PBS up to 10 mL total volume**.
4. Mix gently by inversion; **do not vortex**.

This 2× buffer is diluted 1:1 with PBS at the time of digestion (2 mL 2× buffer + 2 mL PBS per liver sample).

Storage and stability

- Store freshly prepared digestion buffer **on ice** during use.
- Do **not** freeze after mixing with enzymes.
- Discard after same-day use.
- Collagenase IV and DNase I **stock solutions**:
 - Store at **−20 °C**, **aliquoted**, avoid repeated freeze–thaw cycles.

Reagent details

| Reagent | Supplier | Cat. # | Notes |
|----------------|----------|--------|------------------------|
| Collagenase IV | Various | — | Liver digestion enzyme |
| DNase I | Various | — | Prevents clumping |
| PBS, sterile | Various | — | Solvent |

Safety

- Handle enzymes with gloves and eye protection to avoid skin or mucosal irritation.
- Dispose of enzyme-containing waste as biological/chemical waste according to institutional guidelines.
- Use sterile technique inside a biosafety cabinet.

Version history

| Version | Date | Author | Changes |
|---------|------------|----------------|---------------------------------|
| v1.0 | 2025-11-21 | Dillon Corvino | Initial Quarto buffer document. |