Mouse Liver Digestion Buffer

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Protocol ID: BUF-LIV-DIG **Version:** v1.1 **Author:** Dillon Corvino **Date:** 2026-09-18

Purpose

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

This single buffer replaces the former separate Collagenase D and Collagenase IV documents (`BUF-LIV-DIG-COL-D`, `BUF-LIV-DIG-COL-IV`, both retired at v2.0 of `MUS-LIV-DIG`).

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Linked protocols

- `MUS-LIV-DIG`

Choice of collagenase and base medium

Two collagenases are in routine use. They are **not interchangeable at the same concentration** — Collagenase D is used at **twice** the concentration of Collagenase IV.

Enzyme	Working concentration	Notes
Collagenase D	**1 mg/mL**	Roche 11088858001; supplied as lyophilised powder
Collagenase IV	**0.5 mg/mL**	Half the concentration used for Collagenase D

DNase I is 50 ug/mL for both.

The base medium may be **DMEM (incomplete)**, **PBS 1x**, or **HBSS 1x** — these are interchangeable for this application. Use whichever is on hand; HBSS and PBS avoid the phenol red background in DMEM if that matters downstream.

> **!** Important
>
> Do **not** use an EDTA-containing buffer (e.g. MACS Buffer) as the base medium, and do not perfuse the liver with one. Collagenases are Ca2+/Zn2+-dependent metalloproteases and EDTA chelates these cofactors, inhibiting digestion.

Working buffer composition

Enzyme	Final volume (mL)	Base medium	Collagenase (100 mg/mL stock)	DNase I (10 mg/mL stock)
Collagenase D	1	to 1 mL	10 uL	5 uL
Collagenase D	5	to 5 mL	50 uL	25 uL
Collagenase D	10	to 10 mL	100 uL	50 uL
Collagenase D	25	to 25 mL	250 uL	125 uL
Collagenase D	50	to 50 mL	500 uL	250 uL
Collagenase IV	1	to 1 mL	5 uL	5 uL
Collagenase IV	5	to 5 mL	25 uL	25 uL
Collagenase IV	10	to 10 mL	50 uL	50 uL

Enzyme	Final volume (mL)	Base medium	Collagenase (100 mg/mL stock)	DNase I (10 mg/mL stock)
Collagenase IV	25	to 25 mL	125 uL	125 uL
Collagenase IV	50	to 50 mL	250 uL	250 uL

5 mL of working buffer is required per liver.

Preparation

A. Preparation of stock (if applicable)

Reagent	Stock concentration	Storage
Collagenase D or IV	100 mg/mL in sterile PBS	Aliquot at -20 °C
DNase I	10 mg/mL	Aliquot at -20 °C; avoid repeated freeze-thaw

Reconstitution of Collagenase powder. Collagenase D is supplied as a **lyophilised powder** and must be reconstituted before use.

1. Add **1 mL sterile PBS 1x** directly to a vial containing **100 mg** Collagenase powder.
2. Swirl gently until fully dissolved; **do not vortex**.
3. This yields a **100 mg/mL** stock.
4. Aliquot into single-use volumes and store at **-20 °C**, labelled with enzyme, concentration, date, and initials.

B. Preparation of working buffer

1. Thaw collagenase and DNase I aliquots on ice.
2. Add the base medium (DMEM, PBS, or HBSS) to a sterile tube.
3. Add collagenase and DNase I per the table above.
4. Mix gently by inversion; **do not vortex**.
5. Pre-warm to **37 °C** immediately before use.

> **Note**
>
> Collagenase-only buffer (without DNase) may be prepared in advance and frozen in aliquots. Add DNase I fresh on the day of use.

Storage and stability

- Keep freshly prepared working buffer **on ice** until pre-warming for use.
- Do **not** freeze buffer after DNase I has been added.
- Discard unused working buffer at the end of the day.

Reagent details

Component	Supplier	Cat. #	Notes
Collagenase D	Roche, Basel, Switzerland	11088858001	Lyophilised powder
Collagenase IV	[TBD]	[TBD]	
DNase I	Sigma-Aldrich, Merck	DN25-100MG	
DMEM (incomplete)	[TBD]	[TBD]	
HBSS 1x	PAN-Biotech, Aidenbach, Germany	P04-34500	
DPBS, no Ca / no Mg	Gibco, ThermoFisher	14190-094	

Safety (brief)

- Collagenase and DNase I are enzymes of biological origin — handle with standard laboratory PPE and avoid aerosolisation.
- Mouse liver tissue is a biological sample — handle and dispose of according to institutional biosafety rules.
- Dispose of enzyme-containing waste per facility biological/chemical waste procedures.

Version history

Version	Date	Author	Change summary
v1.0	2026-08-05	Dillon Corvino	Created by merging `BUF-LIV-DIG-COL-D` and `BUF-LIV-DIG-COL-IV`. DNase I raised to 50 ug/mL for both enzymes (was 10 ug/mL for Col D, 25 ug/mL for Col IV). Base medium stated as DMEM, PBS, or HBSS.

Version	Date	Author	Change summary
v1.1	2026-09-18	Dillon Corvino	Migrated to shared `summary:`/helper architecture (buffer/protocol redesign); fixed leaked R package-startup warnings in "Linked protocols"; added `# Safety (brief)`; renamed `# Materials` to `# Reagent details`; restructured Preparation into A/B sub-headings.