IEL Strip Medium (DTT/EDTA)

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Protocol ID: BUF-IEL-STRIP **Version:** v1.0 **Author:** Dillon Corvino **Date:** 2026-09-18

Purpose

Used for the DTT/EDTA epithelial stripping step in intraepithelial lymphocyte (IEL) isolation. DTT reduces disulfide bonds in mucus and epithelial junctions; EDTA chelates calcium, destabilising E-cadherin-based junctions — together with mechanical agitation, this releases IELs from the epithelial layer.

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Linked protocols

- `MUS-IEL-ISO`

Working buffer composition

20 mL per mouse, made from 3% Medium (`BUF-IEL-3PCT`) with DTT and EDTA added fresh.

DTT stock: 14.5 mg/mL. MW of DTT = 154.25 g/mol -> 14.5 mg/mL = 94 mM stock. For 1 mM final in 20 mL: add **213 uL** DTT stock.

EDTA stock: 500 mM. For 5 mM final in 20 mL: add **200 uL** EDTA stock.

Component	Final concentration	Volume for 20 mL
3% Medium	Base	19.6 mL
DTT (14.5 mg/mL stock, ~94 mM)	1 mM	213 uL
EDTA (500 mM stock)	5 mM	200 uL

> ⚠ Warning
>
> Prepare fresh and pre-warm to **37 °C** immediately before use. DTT is unstable in solution and loses activity over time. Do not store.

Preparation

A. Preparation of stock (if applicable)

Prepare 3% Medium (`BUF-IEL-3PCT`) in advance — this forms the base. DTT and EDTA stocks may be pre-made and stored (DTT stock at -20 °C; EDTA stock at room temperature), but must only be added to the base medium immediately before use.

B. Preparation of working buffer

1. Add DTT and EDTA stock to 3% Medium per the table above.
2. Mix gently by inversion.
3. Pre-warm to **37 °C** in a water bath immediately before use.

Storage and stability

- Do not store — prepare fresh and use the same day.
- DTT loses activity in solution over time; do not prepare in advance.

Reagent details

Component	Supplier	Cat#	Notes
DTT (dithiothreitol)	[TBD]	[TBD]	Prepare 14.5 mg/mL (~94 mM) stock; add fresh
EDTA (0.5 M stock)	[TBD]	[TBD]	

Safety (brief)

- DTT is a reducing agent with a strong odour; handle in a well-ventilated area and avoid inhalation.
- Standard laboratory PPE otherwise.

Version history

Version	Date	Author	Change summary
v1.0	2026-09-18	Dillon Corvino	Split out of `prot_mus_iel_isolation.qmd` into its own buffer document (buffer/protocol architecture redesign) — no change to composition.