Mouse Small Intestinal Intraepithelial Lymphocyte (IEL) Isolation

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Purpose

This protocol describes the isolation of **intraepithelial lymphocytes (IELs)** from the mouse small intestine for downstream flow cytometry. IELs reside within the intestinal epithelial layer, intercalated between epithelial cells, and are physically distinct from lamina propria lymphocytes (LPLs).

The protocol uses DTT/EDTA-based epithelial stripping combined with mechanical agitation to release the epithelial fraction, followed by Percoll density centrifugation to enrich lymphocytes. An optional ACK RBC lysis step is included.

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Prep checklist

Everything below is **per mouse**. Multiply by the number of animals, and add margin on the buffers so the last sample is not short.

Reagents

Reagent	Per mouse	Where it is used
3% Medium	**50 mL**	STEP 2 intestine washes (20 mL) + base for Strip Medium (20 mL) + 10 mL margin
Strip Medium	**20 mL**	STEP 3 — **make fresh and pre-warm to 37 °C**; DTT is unstable in solution
Shake Medium	**25 mL**	STEP 4 — 2 x 10 mL shake rounds + 5 mL margin
35% Percoll	**10 mL**	STEP 6
PBS	**~60 mL**	STEP 2 wash, STEP 6 post-Percoll wash, STEP 7 top-up to 30 mL, STEP 8 resuspension
ACK lysis buffer	**7 mL**	STEP 7 — optional; often omitted after Percoll

> ⚠ Warning
>
> Strip Medium is the only buffer that **must** be made on the day — DTT loses activity in solution. 3% Medium and Shake Medium can be prepared the evening before and held at 4 °C.

Plasticware and consumables

Item	Per mouse	Note
50 mL Falcon tube	**4**	Transport (STEP 2), shake rounds (STEP 4), pooled strip + shake fractions (STEP 5), fresh tube post-ACK (STEP 7). **3** if ACK is omitted
Beaker + magnetic stir bar	**1**	STEP 3 stripping — see the note below
70 um cell strainer	**1**	STEP 7, post-ACK filtration
15 mL conical tube	**1, optional**	Only if running the Percoll step in a smaller volume
Aluminium foil	1 piece	Covers the beaker during stripping
Transfer pipette	**1**	Resuspension steps

> **!** Important
>
> The **20 minute stripping step at 37 °C is the throughput bottleneck**. One beaker and stir bar are needed per mouse being stripped simultaneously — if the heated stir plate holds only 2–3 beakers, samples must be staggered in rounds. Count the available positions before planning the day.

Shared, not per mouse: metal strainer and large collection beaker (wash thoroughly between mice), 37 °C heated magnetic stir plate, 37 °C water bath, refrigerated centrifuge with adjustable brake, fine scissors, forceps, ice, timers.

Background: gut anatomy, immune compartments, and isolation logic

This section provides an anatomical and immunological reference for the intestinal tract relevant to IEL isolation. It is intended as orientation for researchers new to gut immunology and as a practical guide for experimental design decisions (segment choice, compartment targeting, downstream assay selection).

Overview of the intestinal tract

The mouse intestinal tract runs approximately 30 cm in total and is divided into the **small intestine** and **large intestine**, each with distinct anatomy, function, and immune composition.

Small intestine

The small intestine comprises three contiguous segments, running proximal to distal:

Segment	Approximate proportion	Key anatomical features	Primary function
Duodenum	~5% of SI length	Short; receives bile and pancreatic secretions; long villi (villus:crypt ~3–5:1)	Digestion of fats, carbohydrates, protein; iron absorption
Jejunum	~40% of SI length	Longest segment; most active absorption; longest and densest villi	Primary nutrient absorption
Ileum	~55% of SI length	Shorter villi; highest density of Peyer's patches; terminal segment before cecum	Vitamin B12 and bile acid reabsorption; immune sampling

The small intestinal epithelium is organised into **finger-like villi** projecting into the lumen (absent in the large intestine) and **crypts of Lieberkühn** at the base of the villi. This villus–crypt architecture dramatically increases luminal surface area and is the structural context within which IELs reside.

Large intestine

The large intestine follows the small intestine and comprises:

Segment	Notes
Cecum	Blind-ended pouch at the SI–LI junction; largest microbial reservoir; enlarged in germ-free mice
Colon (proximal → distal)	Lacks villi; has crypts only; much thicker mucus layer than SI; primary site of water reabsorption
Rectum / anus	Terminal segment

The large intestine has a far higher microbial load than the small intestine (bacterial density increases ~1,000-fold from duodenum to colon) and a correspondingly distinct immune environment dominated by regulatory and microbiota-responsive cells.

> **Note**
>
> IEL density is substantially higher in the small intestine than the large intestine. The large intestine has approximately 5–10× fewer IELs per epithelial cell than the small intestine under steady-state conditions. This protocol targets **small intestinal IELs only**.

Wall architecture and immune compartments

The intestinal wall is organised into concentric layers. Moving from the lumen outward:

```
Lumen


Epithelium          ← IELs live here (within the epithelial layer)

Basement membrane

Lamina propria      ← LPLs, DCs, macrophages, mast cells, ILCs

Muscularis mucosae

Submucosa           ← Nerve plexus, blood and lymphatic vessels

Muscularis externa  ← Smooth muscle (inner circular + outer longitudinal)

Serosa / mesentery
```

For immunological purposes, the two most important layers are the epithelium and the lamina propria.

Epithelial layer and the IEL compartment

The intestinal epithelium is a **single-cell-thick monolayer** of epithelial cells (primarily enterocytes) that lines the entire luminal surface. IELs are embedded within this monolayer, sitting between neighbouring epithelial cells with their cell bodies typically below the tight junctions but above the basement membrane. They are in direct contact with the epithelium and sample the luminal environment.

In the small intestine, there is approximately **1 IEL per 5–10 epithelial cells**, making the gut epithelium one of the largest T cell compartments in the body by cell number. IELs are long-lived, largely non-recirculating tissue-resident cells.

How to access IELs: Because IELs are mechanically held within the epithelial layer by integrin interactions (primarily E 7/CD103), they are released by disrupting epithelial junctions with EDTA (chelates calcium, destabilising E-cadherin-based junctions) and DTT (reduces disulfide bonds in mucus), combined with mechanical agitation. This is the stripping step in this protocol.

Lamina propria and the LPL compartment

The lamina propria is the loose connective tissue layer immediately beneath the epithelium, separated from it by the basement membrane. It contains:

- **T cells** — predominantly CD4+ T helper cells; also CD8+ effector T cells; Tregs
- **B cells and plasma cells** — IgA-secreting plasma cells are abundant; responsible for secretory IgA production
- **Dendritic cells** — CD11b+CD8 − DCs predominate in the small intestinal LP; important for antigen sampling and T cell priming
- **Macrophages** — major phagocytic and tolerogenic population; form close contacts with the epithelium
- **Mast cells** — involved in helminth responses and barrier defence
- **Innate lymphoid cells (ILCs)** — ILC1, ILC2, ILC3 populations present; ILC3s produce IL-22 for barrier maintenance

How to access LPLs: The basement membrane must be disrupted after removing the epithelial layer. This requires **enzymatic digestion** of the remaining tissue with collagenase (± DNase), typically at 37 °C with agitation. LPL isolation is a separate workflow from IEL isolation and is not covered in this protocol. The intestinal pieces remaining after the stripping and shake steps in this protocol can be used directly as input for LP digestion.

Peyer's patches

Peyer's patches are **organised lymphoid follicles** embedded in the intestinal wall, predominantly in the ileum. They are visible as pale, dome-shaped nodules on the anti-mesenteric surface. They contain:

- Dense **B cell follicles** (B220+ B cells)
- **Germinal centre** reactions for IgA class switching
- **T cell zones** with CD4+ T cells
- **Specialised M cells** (microfold cells) in the overlying follicle-associated epithelium for antigen transcytosis

Peyer's patches are **not part of the IEL or LP compartments** and must be removed before processing. If retained, the B cell-rich follicular content heavily contaminates IEL preparations and distorts flow cytometry data.

Segment-specific immune cell composition

Small intestinal IEL subsets by segment

IEL subset distribution is not uniform along the small intestine. The two major functional categories of IELs are:

- **Natural IELs (nIELs)** — arise from thymic precursors; present in germ-free mice; include TCR + IELs and TCR + CD8 + IELs. More abundant in the **proximal SI (duodenum/jejunum)**.
- **Peripheral/induced IELs (pIELs)** — arise from conventional peripheral T cells that down-regulate CD8 and upregulate CD8 (or become CD4+CD8 +) upon entry into the epithelium. Enriched in the **ileum**, driven by microbiota-dependent signals including high MHC-II and PD-L1 expression in that region.

IEL subset	TCR	CD8 form	Segment enriched	Microbiota-dependent?
nIEL type a	TCR	CD8 or DN	Proximal SI	No (present in GF)
nIEL type b	TCR	CD8	Proximal SI	No (present in GF)
pIEL (induced)	TCR	CD4+CD8 +	Ileum	Yes (absent/low in GF)
pIEL (induced)	TCR	CD8 → CD8	Ileum	Yes

For most standard CD8 IEL analyses, the **whole small intestine** is used to maximise yield. Segment-specific harvest is appropriate only when subset enrichment is the experimental goal.

Lamina propria immune cell composition by segment

LP immune composition also differs significantly by segment:

Cell type	Proximal SI (duodenum/jejunum)	Ileum	Colon
CD4+ T cells (Th1/Th2)	Present	Present	Present
Th17 cells	Low	**Enriched** (SFB-driven in SPF mice)	Moderate
Tregs	Present	Present	**Enriched**
IgA plasma cells	Moderate	High	**Highest**
DCs	CD11b+CD8 − predominate	CD11b+CD8 −	Fewer DCs in homeostasis
Macrophages	Present	Present	Present; recruited upon inflammation
ILC3 / IL-22 producers	Present	Present	Present

Large intestinal immune environment

The colon has a distinct immune composition compared with the small intestine:

- **Thicker, stratified mucus layer** — creates a physical exclusion zone separating bacteria from the epithelium; much more prominent than in SI.
- **Higher regulatory T cell frequency** — the colon is more tolerogenic overall.
- **Microbiota-dependent immune cell shaping** — colonic immune cell composition is more severely disrupted in germ-free conditions than SI.
- **Fewer IELs and DCs** — under homeostatic conditions; both are recruited upon inflammation.
- **No villi** — crypts only; different epithelial cell organisation affects IEL numbers and accessibility.

How isolation strategy maps to compartment

Target population	Compartment	Isolation strategy	When to use
IELs	Epithelium	EDTA/DTT stripping + mechanical agitation → Percoll	This protocol
LPLs	Lamina propria	Enzymatic digestion (collagenase/DNase) of post-strip tissue	Separate LP protocol
Peyer's patch cells	Organised follicles	Mechanical dissociation through strainer (separate from SI processing)	Lymphoid follicle studies
MLN cells	Mesenteric lymph nodes	Mechanical dissociation through strainer	Draining lymph node studies
Combined IEL + LP	Both	Sequential: strip first → digest remainder	Full mucosal immune profiling

> **Note**
>
> This protocol uses the **whole small intestine** as the default. If segment-specific enrichment is required (e.g. ileum only for pIEL studies, or proximal SI for nIEL studies), dissect and process each segment separately. Label clearly: duodenum begins immediately distal to the pyloric sphincter; the ileum ends at the ileocecal junction; the jejunum is the long intervening segment.

Critical notes (read before starting)

- Remove **all Peyer's patches** before processing — they are B-cell-rich and will contaminate the IEL fraction if retained.
- **Open the intestine longitudinally** and wash thoroughly before stripping — residual luminal contents and mucus inhibit stripping efficiency and reduce recovery.
- DTT reduces disulfide bonds in mucus and epithelial junctions, improving IEL release; **do not exceed recommended concentration or incubation time** as overdose can reduce viability.
- The **stripping step uses a magnetic stirrer at 37 °C** — this is a key feature of this protocol and improves IEL yield compared with passive incubation.
- The **manual shake step** after stripping further mechanically dislodges IELs; perform it vigorously.
- **Percoll enrichment is essential** — without it, the preparation will be heavily contaminated with epithelial cells.
- Process samples promptly; keep samples on ice between active steps.

Approximate timing

- Intestine harvest, opening, and washing: **15–20 min per mouse**
- DTT/EDTA stripping (37 °C stir): **20 min**
- Manual shake steps (×2): **5 min**
- Centrifugation and Percoll enrichment: **40–50 min**
- ACK lysis, wash, and final resuspension: **15 min**

Total approximate time: ~1.5–2 hours per mouse (batching 2–4 mice in parallel is feasible with practice)

Procedure

STEP 1 – Preparation and setup

1. Prepare all buffers fresh (see Buffers section). Pre-warm **Strip Medium** to **37 °C** in a water bath. Keep all other buffers on ice.
2. Set up a **magnetic stir plate at 37 °C** with a stir bar and a beaker — this is used for the stripping step.
3. Prepare a **metal strainer** over a large collection beaker for filtering after stripping.
4. Label 50 mL tubes for each mouse: one for stripping collection, one for downstream processing.
5. Pre-cool the centrifuge to **4 °C**.
6. Prepare **35% Percoll working solution** (see Buffers section) at room temperature.

STEP 2 – Intestine harvest and preparation

1. Euthanise the mouse by an approved method.
2. Open the abdominal cavity and locate the small intestine.
3. Excise the **entire small intestine**, cutting just distal to the pyloric sphincter (stomach) and at the ileocecal junction.
4. Remove all attached **mesenteric fat and connective tissue** along the intestinal length.
5. Identify and excise all **Peyer's patches** (pale, dome-shaped lymphoid nodules on the anti-mesenteric border). Cut them away and discard.

> **Warning**
>
> Peyer's patches must be removed completely. They contain a distinct lymphoid population that will contaminate the IEL fraction.

6. Cut the intestine **open longitudinally** along its entire length using fine scissors.
7. **Wash the opened intestine once with 3% Medium** and then **once with PBS** to remove luminal contents and mucus.
8. After washing, transfer the intestinal tissue to a 50 mL Falcon tube with a small volume of 3% Medium to keep it moist until the next step.

> **Note**
>
> Thorough washing after opening is critical. Residual mucus reduces stripping efficiency.

STEP 3 – DTT/EDTA stripping (37 °C magnetic stir)

1. Cut the washed intestinal tissue into approximately **5 pieces** of roughly equal length.
2. Place the pieces into the pre-warmed **beaker** (on the magnetic stir plate at 37 °C) containing **20 mL Strip Medium** (RPMI/3% FCS + 1 mM DTT + 5 mM EDTA, pre-warmed to 37 °C).
3. Add a **magnetic stir bar** to the beaker. Cover with **aluminium foil**.
4. Incubate at **37 °C for 20 minutes** with continuous stirring at **250 rpm**.

> ⚠ Warning
>
> Do not exceed the 20-minute incubation or increase DTT concentration above 1 mM. Over-exposure to DTT reduces leukocyte viability.

5. After incubation, strain the contents through the **metal strainer** into the collection beaker to collect the stripped epithelial fraction.
6. Keep the intestinal pieces on the strainer for the manual shake step (STEP 4).

STEP 4 – Manual shake washes

The mechanical shake step dislodges residual IELs from the tissue and maximises recovery.

Shake round 1

1. Transfer the intestinal pieces from the metal strainer into a **50 mL Falcon tube** containing **10 mL Shake Medium** (RPMI + 1% glutamine + 1% Pen/Strep + 2% HEPES + 2 mM EDTA).
2. Cap the tube and **shake vigorously by hand for 30 seconds**.
3. Pour the tube contents back through the **metal strainer** into the collection beaker.

Shake round 2

1. Return the intestinal pieces to the Falcon tube with a fresh **10 mL Shake Medium**.
2. Shake vigorously for 30 seconds as before.
3. Pour back through the **metal strainer** into the collection beaker.

The collection beaker now contains the pooled stripping and shake fractions. The intestinal tissue pieces can be discarded or retained for LP isolation.

STEP 5 – Transfer and centrifugation

1. Transfer the total cell suspension from the collection beaker into a **50 mL Falcon tube**.
2. Centrifuge at **400 g, 4 °C, 10 minutes**.

> ℹ Note
>
> The colleague protocol reports 550 g for this step. Standard published IEL protocols use 400 × g for this wash spin. Confirm with your local centrifuge rotor to determine the exact rpm corresponding to your instrument settings.

3. Discard the supernatant.

STEP 6 – 35% Percoll enrichment

1. Resuspend the pellet in **10 mL of 35% Percoll**.

> **Note**
>
> **On Percoll percentage:** This protocol uses **35% Percoll** as per the collaborator protocol. Literature precedent for mouse IEL isolation varies: single-density pellet-through protocols typically use **35–44%**, while discontinuous gradient protocols use **44%/67%** (interface collection) for higher purity. The 44/67% discontinuous gradient consistently yields 30–80% CD45+ lymphocytes post-separation and is used in the most widely cited murine IEL protocols (Lefrancois & Lycke 2001; Bhatt & Bhatt 2022 PMC8128104). A 37% single-density pellet-through (the concentration used in the lab for liver) should also work for IEL and is compatible with this workflow; lymphocytes pellet while many epithelial cells and debris remain in the supernatant. If purity is insufficient with 35–37%, consider switching to the 44/67% discontinuous gradient approach.

2. Centrifuge at **800 g, 25 minutes, room temperature (20–22 °C)**:
    - Acceleration: **moderate (acc 7)**
    - Brake: **minimal (br 1)**

> **Warning**
>
> Do not use high brake — abrupt deceleration disrupts the interface and reduces enrichment.

3. After centrifugation:
    - **Lymphocytes pellet** at the bottom.
    - Epithelial cells, debris, and dead cells remain in the supernatant.
4. Carefully aspirate and discard the supernatant in one smooth motion.
5. Wash the pellet: resuspend in **PBS**, centrifuge at **400 g, 4 °C, 10 minutes**. Discard supernatant.

STEP 7 – ACK RBC lysis (optional)

> **Note**
>
> RBC contamination in IEL preparations is generally low because the intestinal epithelial compartment contains few red blood cells. This step can often be omitted, particularly if a Percoll gradient has been performed. Include if the pellet appears visibly red.

1. Resuspend the cell pellet in **7 mL ACK lysis buffer**.
2. Incubate for approximately **10 minutes at room temperature**.
3. Pass the suspension through a **70 µm cell strainer** into a fresh 50 mL tube.
4. Top up to **30 mL with PBS**.
5. Centrifuge at **400 g, 4 °C, 10 minutes**.
6. Discard the supernatant.

STEP 8 – Final resuspension and staining

1. Resuspend the cell pellet in **300 µL PBS**.
2. Count cells and assess viability as required.
3. Use **100 µL of suspension per flow cytometry panel**.

4. Add **100 µL antibody master mix** and stain for **20 minutes** (on ice, protected from light, as per staining SOP).
5. After staining, resuspend in **200 µL PBS** for acquisition.

> Tip
>
> Expected yield from one small intestine: approximately **3–6 × 10^6 total IEL** from an 8–12-week-old naïve C57BL/6 mouse after Percoll enrichment (literature estimate; actual yield depends on mouse age, strain, and protocol execution). Contaminating epithelial cells can be excluded in flow cytometry using **EpCAM (CD326)** in the dump channel.

Buffers Required

Full composition, preparation, storage, and safety information for each buffer lives in its own linked buffer document — only a concise per-buffer summary is reproduced here, generated automatically from that document.

> **Note**
>
> The 35% Percoll working solution used in this protocol is diluted from the same **isotonic Percoll stock** as `BUF-PERC-37` (the 37%-of-isotonic-stock buffer used for liver digestion), just to a different final percentage (35% vs 37%). See `BUF-PERC-37` for isotonic stock preparation; the summary below states the 37% working dilution, not the 35% variant used here — for the 35% dilution use 3.5 mL isotonic Percoll + 6.5 mL PBS per 10 mL.

3% Medium (IEL Wash/Base Medium)

Component	Final concentration / amount
RPMI 1640	46.0 mL (base)
FCS	1.5 mL (3% v/v)
HEPES (1 M stock)	1.0 mL (2% v/v)
Penicillin/Streptomycin (100x stock)	0.5 mL (1% v/v)
L-glutamine (200 mM stock)	0.5 mL (1% v/v)

- **Final volume:** 50 mL per mouse
- **Storage:** Can be prepared the evening before and held at 4C.

Full composition, preparation steps, storage, and safety information: ***`BUF-IEL-3PCT`****.*

IEL Strip Medium (DTT/EDTA)

Component	Final concentration / amount
3% Medium (BUF-IEL-3PCT)	19.6 mL (base)
DTT (14.5 mg/mL stock, ~94 mM)	213 uL (1 mM final)
EDTA (500 mM stock)	200 uL (5 mM final)

- **Final volume:** 20 mL per mouse
- **Preparation note:** Prepare fresh and pre-warm to 37C immediately before use.
- **Storage:** Do not store - DTT is unstable in solution and loses activity over time.
- **Caveat:** Do not exceed 1 mM DTT or the 20-minute stripping incubation - over-exposure to DTT reduces leukocyte viability.

Full composition, preparation steps, storage, and safety information: ***`BUF-IEL-STRIP`****.*

IEL Shake Medium

Component	Final concentration / amount
RPMI 1640	23.6 mL (base)
L-glutamine (200 mM stock)	250 uL (1% v/v)
Penicillin/Streptomycin (100x stock)	250 uL (1% v/v)
HEPES (1 M stock)	500 uL (2% v/v)
EDTA (500 mM stock)	100 uL (2 mM final)

- **Final volume:** 25 mL per mouse
- **Storage:** Can be prepared the evening before and held at 4C.

Full composition, preparation steps, storage, and safety information: `BUF-IEL-SHAKE`.

Percoll Solution (37% of isotonic stock = 33% final)

Component	Final concentration / amount
Percoll (isotonic)	9.25 mL (37% of isotonic stock = 33% final Percoll)
PBS 1x	15.75 mL

- **Final volume:** 25 mL per mouse liver
- **Preparation note:** Equilibrate isotonic Percoll stock to room temperature before diluting; mix gently by inversion. Prepare fresh on the day of use.
- **Storage:** Room temperature, same-day use only; do not freeze working solution
- **Caveat:** 37% (of isotonic stock) and 33% (final Percoll) both describe this same solution, just measured against different denominators - check which convention an external protocol uses before changing any number.

Full composition, preparation steps, storage, and safety information: `BUF-PERC-37`.

ACK RBC Lysis Buffer

Component	Final concentration / amount
Ammonium chloride (NH4Cl)	8.26 g/L
Potassium bicarbonate (KHCO3)	1.00 g/L
EDTA	0.037 g/L (37 mg/L)

- **Final volume:** 1 L
- **Preparation note:** Dissolve in sterile distilled water, then adjust to pH 7.2 with dilute HCl before sterile filtration.
- **Sterilisation / filtering:** 0.22 um filter required
- **Storage:** 4C; stable up to 3 months when sterile; do not freeze.

Full composition, preparation steps, storage, and safety information: `BUF-ACK-LYSIS`.

Materials

Reagents

Reagent	Supplier / Cat#	Notes
RPMI 1640	Various	Base for all three buffers
FCS	Various	3% in 3% Medium and Strip Medium
HEPES (1 M stock)	Various	2% v/v final
L-glutamine (200 mM stock)	Various	1% v/v final
Penicillin/Streptomycin (100×)	Various	1% v/v final
DTT (dithiothreitol)	Various	Prepare 1 M stock in water; add fresh to Strip Medium
EDTA (0.5 M stock)	Various	5 mM in Strip Medium; 2 mM in Shake Medium
Percoll (isotonic stock)	Cytiva / GE	See `BUF-PERC-37` for isotonic prep
PBS 1× (Ca^2 /Mg^2 -free)	Various	For washes
ACK lysis buffer	Commercial or in-house	Optional RBC lysis

Disposables

Item	Notes
50 mL Falcon tubes	Multiple per mouse
15 mL conical tubes	Optional, for Percoll if using smaller volume
Metal strainer	For stripping and shake washes
Collection beaker	For pooling fractions
40 µm cell strainer	For filtering post-ACK lysis
Fine scissors	For opening intestine longitudinally
Forceps	For tissue handling
Magnetic stir bar	For 37 °C stripping step
Aluminium foil	To cover beaker during stripping
Transfer pipettes	For resuspension and transfers

Equipment

Equipment	Notes
Magnetic stir plate with heating	37 °C, 250 rpm — critical for stripping step
Water bath (37 °C)	Pre-warming Strip Medium
Refrigerated centrifuge	Compatible with 50 mL tubes; adjustable brake
Biosafety cabinet (Class II)	Recommended
Ice bucket	Keep samples cold between steps
Timer	For stripping and lysis timing

Troubleshooting

Problem	Possible cause	Suggested solution
Low IEL yield	Incomplete intestinal opening or washing; insufficient stirring speed	Open intestine fully; wash thoroughly before stripping; confirm stir plate is at 250 rpm
High epithelial cell contamination	Percoll % too low; lysis insufficient	Consider increasing to 40–44% Percoll single density or switching to 44/67% discontinuous gradient; use EpCAM dump in flow panel
Poor viability	DTT over-exposure; prolonged room-temperature handling	Do not exceed 20 min stripping or 1 mM DTT; keep post-stripping steps cold
Mucus clumping / strainer blockage	Insufficient pre-wash	Increase washing steps after intestinal opening; DTT helps but is not a substitute for mechanical mucus removal
Peyer's patch contamination	Incomplete removal	Systematically inspect anti-mesenteric border and excise all visible nodules before stripping
Red pellet after Percoll	RBC contamination (uncommon for IEL)	Perform ACK lysis step (STEP 7)
Low purity (few CD45+ cells)	35% Percoll insufficient for separation	Trial 40–44% single gradient or 44/67% discontinuous gradient

Notes on Percoll percentage and protocol variants

35% vs 37% vs 40–44% for IEL isolation

The Percoll concentration used for IEL enrichment varies across published protocols:

- **35%** — used by some groups (including this collaborator protocol) as a single-density pellet-through. Lymphocytes pellet; many epithelial cells remain in the supernatant. Simpler but lower purity.
- **37%** — the lab standard for liver leukocyte enrichment (`BUF-PERC-37`). Compatible with IEL isolation and can be used as a practical alternative to 35% without changing the isotonic Percoll stock preparation.
- **40–44%** — more widely used in published murine IEL protocols. Higher Percoll density improves separation of lymphocytes from epithelial cells and increases preparation purity.
- **44%/67% discontinuous gradient** — the gold standard in the most widely cited protocols (Lefrancois & Lycke 2001; Bhatt & Bhatt 2022). IELs are collected from the 44/67% interface rather than as a pellet, yielding 30–80% CD45+ purity. Requires underlaying 67% Percoll and more careful technique but gives the cleanest preparations.

Practical recommendation: Start with 35% (as in this protocol, matching the collaborator method). If purity is insufficient for your flow panel or gating (assess by EpCAM vs CD45 plot), escalate to a 44/67% discontinuous gradient.

nIELs vs pIELs and segment choice

IEL subset	Segment enriched	Notes
nIELs (TCR , CD8 TCR)	Duodenum / proximal jejunum	More abundant proximally in SPF mice
pIELs (CD4+CD8 +)	Ileum	Driven by microbial signals; highest in distal SI
Total IEL yield	Whole small intestine	Standard for most panels; maximises cell numbers

Safety (brief)

- All animal procedures must comply with approved animal licences and institutional regulations.
- Wear appropriate PPE (lab coat, gloves, eye protection) when handling animals, tissues, and reagents.
- DTT is a reducing agent with a strong odour; handle in a well-ventilated area and avoid inhalation.
- Dispose of biological waste and sharps according to institutional biosafety guidelines.

Version history

Version	Date	Author	Change summary
v1.0	2026-03-19	Dillon Corvino	Initial IEL isolation protocol for small intestine; adapted from collaborator protocol with literature integration
v1.1	2026-03-20	Dillon Corvino	Added comprehensive gut anatomy and immune compartment reference section
v1.2	2026-04-08	Dillon Corvino	Moved TOC to after Purpose; resolved g-force placeholders (400 g); updated Step 5 note to 550 g; replaced 40 µm with 70 µm strainer; added per-mouse buffer volumes with stock calculations for DTT and EDTA

Version	Date	Author	Change summary
v1.3	2026-09-18	Dillon Corvino	Removed the two duplicate inline `# Buffers` sections (full recipes were previously hand-copied here). 3% Medium, Strip Medium, and Shake Medium split out into their own buffer documents (`BUF-IEL-3PCT`, `BUF-IEL-STRIP`, `BUF-IEL-SHAKE`); added missing `BUF-ACK-LYSIS` dependency. Replaced with an auto-generated `# Buffers Required` section (one heading + one small table per buffer, sourced from each buffer's own `summary:` data). Fixed stale `BUF-PERC-37-001` references to `BUF-PERC-37`.