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Protocol status: Working We use this protocol and it's working

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Gut prep of intestinal immune cells

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ABSTRACT

This protocol details the procedure of purification of immune cells from the mouse small intestine.

ATTACHMENTS

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MATERIALS

Materials

- PBS
- paper towel
- conical tube
- HBSS
- EDTA
- HEPES
- FBS
- RPMI
- DNase I
- Dispase (5U/mL)
- Collagenase D (Roche)
- falcon tube
- Percoll

Last Modified: Jul 16, 2023

PROTOCOL integer ID: 84893

Keywords: gut, lamina propria, immune cell, small intestine

	Procedure	57m 55s
1		
	Note	
	Note : This protocol is adapted from Ivanov et al, 2006.	
	Cut out the entire small intestine – from stomach to caecum.	
	Note	
	As you are cutting it out, remove residual fat and connective tissue.	
2	Place in ice cold PBS, move on to the next mouse.	
3	Continue removing all fat and connective tissue.	
4	Cut out the Peyer's patches.	
	Note	
	There should be 9-12 PPs in total.	
5	Cut open the intestine longitudinally.	

- 6 Place on a wet paper towel, use rounded forceps to scrape along the mucosa, removing mucous, bacteria, etc.
- 7 Place in a 50 mL conical tube with PBS, invert tube a few times.
- **8** Pour of the supernatant, refill with fresh PBS.
- **9** Repeat the PBS washes 5-6 more times until there is no visible debris.



- 10 Cut the intestine in large fragments.
- Place the fragments in a 15 mL conical tube with $\boxed{\pm}$ 5-10 mL cell dissociation solution.
- 11.1 To make 120 mL of cell dissociation solution (adjust as necessary):

A	В
HBSS	114.5 mL
0.5 M EDTA	1.2 mL
1 M HEPES	1.2 mL
FBS	3.12 mL

12

Incubate for \$\(\(\omega \) \frac{100 \text{ rpm, } 37^\circ C,}{00:10:00} \)

on the rotator.

Vortex well for 00:00:25, take out the supernatant with a metal strainer, keep the pieces, discard the supernatant.

25s



- **14** Repeat steps 10-12.
- Collect the fragments, rinse in HBSS in a small petri dish, cut using a razor blade (~1 mm²).
- Digest for 00:20:00 at 37 °C with slow rotation in 5 mL digestion mix.

20m



16.1 To make 4 180 mL of digestion mix (adjust as necessary):

A	В
RPMI	126.6 mL
FBS	9 mL
DNase I	720 ul
Dispase (5U/mL)	18 mL
Collagenase D (Roche)	360 ul

From \$\textsup 500 mg/mL stock solution, to make working conc of \$\textsup 1 mg/mL\$

17 Vortex well for (00:00:30

30s

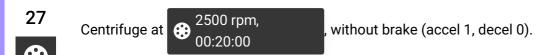


- Collect the supernatant by filtering through $+1+100~\mu m$ strainer in 50 mL falcon tube at Room temperature to avoid cold temperature shock. Keep it 8 On ice afterwards.
- Put remaining tissue fragment back into the same tube with 45 mL of new digestion mix.
- Repeat steps 16-18 two more times.
- 21 Combine all appropriate supernatants.
- Centrifuge at 3000 rpm, 4°C, 00:10:00
 - 00:10:00
- 23 Prepare Percoll (adjust amounts as necessary).
 - a. 100% Percoll = 🔼 45 mL stock Percoll + 🗘 5 mL 10X PBS (🚨 50 mL total).
 - b. 80% Percoll = $\boxed{\bot}$ 24 mL 100% Percoll + $\boxed{\bot}$ 6 mL 10% RPMI-FBS ($\boxed{\bot}$ 30 mL total).
- Add <u>A 5 mL</u> of 80% Percoll to the bottom of a 15 mL conical tube.



Resuspend with LPL cells in $\boxed{ \bot 1 \text{ mL} }$ of 40% Percoll to get homogenous solution, then add the remaining $\boxed{ \bot 9 \text{ mL} }$.

10m



20m

- Collect and discard the top layer of epithelium and debris.
- 29 Collect white cell layer at the interface between the 40% and 80% Percoll.
- 30 Dilute with 10% RPMI-FBS, invert a few times to mix well.
- Pellet by centrifugation for Pellet by centrifugation for 00:07:00

7m

32 Aspirate SN and resuspend cells FACS buffer, then start FACS stain.