Gut prep of intestinal immune cells

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MANUSCRIPT CITATION: Ivanov, II, McKenzie, B.S., Zhou, L., Tadokoro, C.E., Lepelley, A., Lafaille, J.J., Cua, D.J., and Littman, D.R. (2006). The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 126, 1121-1133.

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

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ABSTRACT

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

ATTACHMENTS

786-1999.pdf

MATERIALS

Materials

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

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Procedure

57m 55s

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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.

11 Place the fragments in a 15 mL conical tube with 5-10 mL cell dissociation solution.

11.1 To make 120 mL of cell dissociation solution (adjust as necessary):

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

12 Incubate for 100 rpm, 37°C, 00:10:00 on the rotator.

13 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.

15 Collect the fragments, rinse in HBSS in a small petri dish, cut using a razor blade (~1 mm^2).

16 Digest for 00:20:00 at 37 °C with slow rotation in 5 mL digestion mix. 20m

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

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

From 500 mg/mL stock solution, to make working conc of 1 mg/mL.

17 Vortex well for 00:00:30. 30s

18 Collect the supernatant by filtering through 100 µm strainer in 50 mL falcon tube at Room temperature to avoid cold temperature shock. Keep it On ice afterwards.

19 Put remaining tissue fragment back into the same tube with 5 mL of new digestion mix.

20 Repeat steps 16-18 two more times.

21 Combine all appropriate supernatants.

22 Centrifuge at 3000 rpm, 4°C, 00:10:00 . 10m

23 Prepare Percoll (adjust amounts as necessary).

a. 100% Percoll = 45 mL stock Percoll + 5 mL 10X PBS (50 mL total).

b. 80% Percoll = 24 mL 100% Percoll + 6 mL 10% RPMI-FBS (30 mL total).

c. 40% Percoll = 20 mL 100% Percoll + 30 mL 10% RPMI-FBS (50 mL total).

24 Add 5 mL of 80% Percoll to the bottom of a 15 mL conical tube.

25 Resuspend with LPL cells in 1 mL of 40% Percoll to get homogenous solution, then add the remaining 9 mL .

26 Gently add 10 mL of the 40% Percoll cell solution on top of the 80% Percoll in the tube.

27 Centrifuge at 2500 rpm, 00:20:00, without brake (accel 1, decel 0). 20m

28 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.

31 Pellet by centrifugation for 2000 rpm, 4°C, 00:07:00. 7m

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