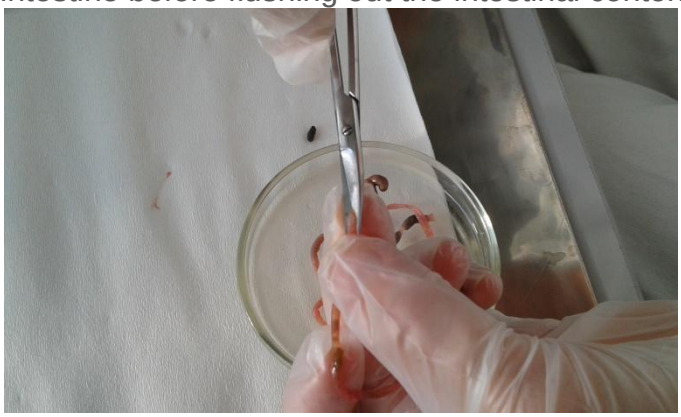
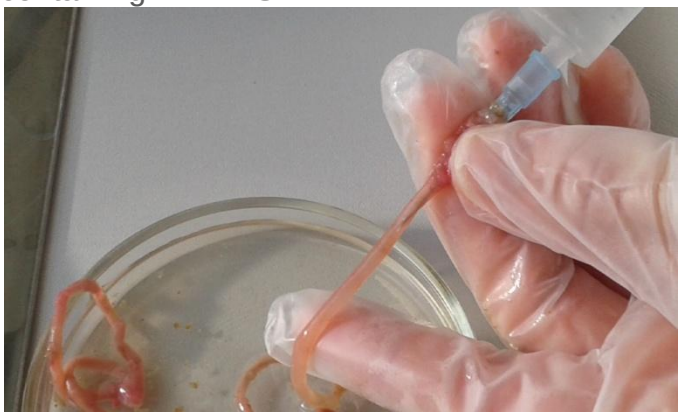
Lamina Propria Lymphocyte Isolation

Method:

1. Remove intestin and put it in a petridish on ice containing 1xDPBS
2. (For small intestine lamina propria preparatum, remove Peyer's patches from small intestine before flushing out the intestinal content.)

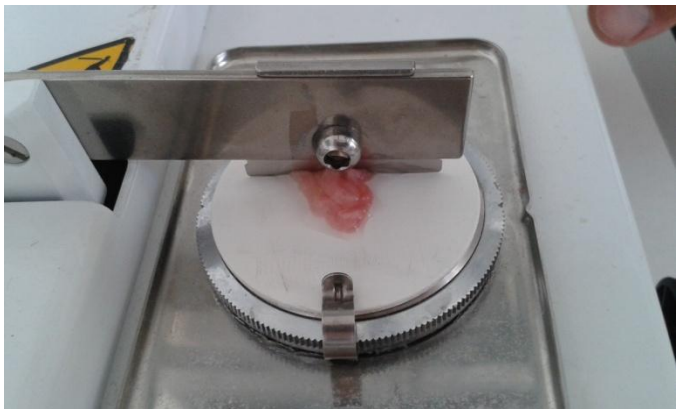


3. Flush out intestinal content using a gavage needle attached to a 10ml syringe containing 1xDPBS.



4. Remove fat tissue from intestine and open longitudinally using a dissection scissor.

5. Put opened intestine back into petridish on ice.
6. Process all mice to this stage and keep the opened intestines on ice in petridish containing 1xDPBS.
7. Cut intestine into 1-3 mm pieces with Mc Ilwain tissue chopper and put them into a 50ml Falcon tube containing 30ml EDTA/HEPES/DPBS solution.

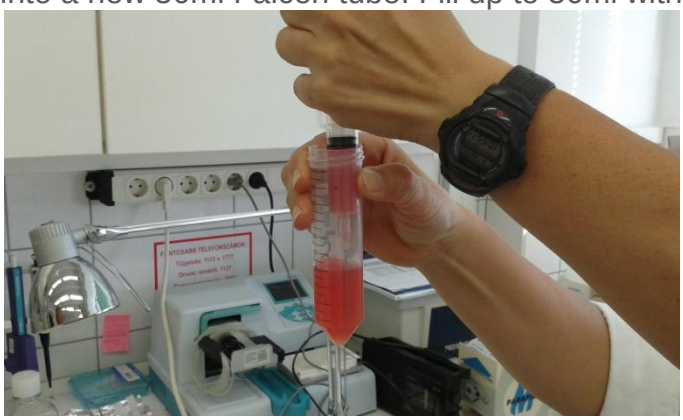


8. For small intestine, only use one small intestine per 50ml Falcon tube containing 30ml EDTA/HEPES/DPBS solution.
EDTA/HEPES/DPBS solution:
- 500ml 1xDPBS **without $MgCl_2$ or $CaCl_2$**
- 5ml 1M HEPES
- 5ml 0.5M EDTA

9. Incubate on shaker at 37oC for 15 min (vigorous shaking ~240rpm). Vortex for 5 sec. Discard supernatant containing epithelium and filter remaining tissue pieces through a 40µm nylon cell strainer. Discard flow through and put retained tissue pieces in a new 50ml falcon tube containing 25ml EDTA/HEPES/DPBS. Repeat these wash steps at least 3 times or until supernatant remains clear after incubation on shaker (epithelium completely removed).
10. Prepare collagenase digestion medium:
- 50ml IMDM
- 0.5ml 1M HEPES
- 25mg collagenase Type VIII (-20°C, 3N42 by scale)
- 1 aliquot DNAse I (-80°C 3N11B, Markus rack)

11. After the last EDTA wash step, transfer retained tissue pieces into a new 50ml Falcon tube containing 25ml digestion medium. Digest for 40-50min on shaker (vigorous shaking ~240rpm).

12. Disaggregate remaining tissue pieces by sucking everything through a pink 18G needle attached to a 20ml syringe. Filter everything through a 100µm nylon cell stariner into a new 50ml Falcon tube. Fill up to 50ml with IMDM.



Spin for 10min at 300 g at 4C. Resuspend the pellet in 10ml IMDM using a 10ml syringe with a pink 18G needle.

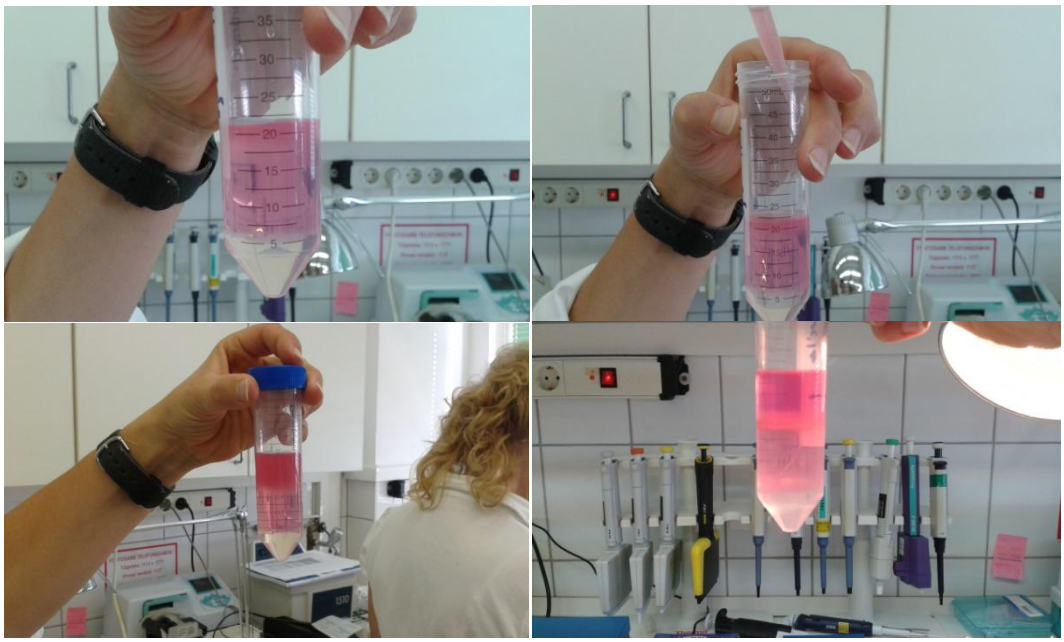
13. Prepare Percoll Gradient (30%/100%): You need the following:

a) osmotic 100% Percoll:

- 45ml Percoll
- 4.5ml 10x HBSS
- 0.5ml 1M HEPES

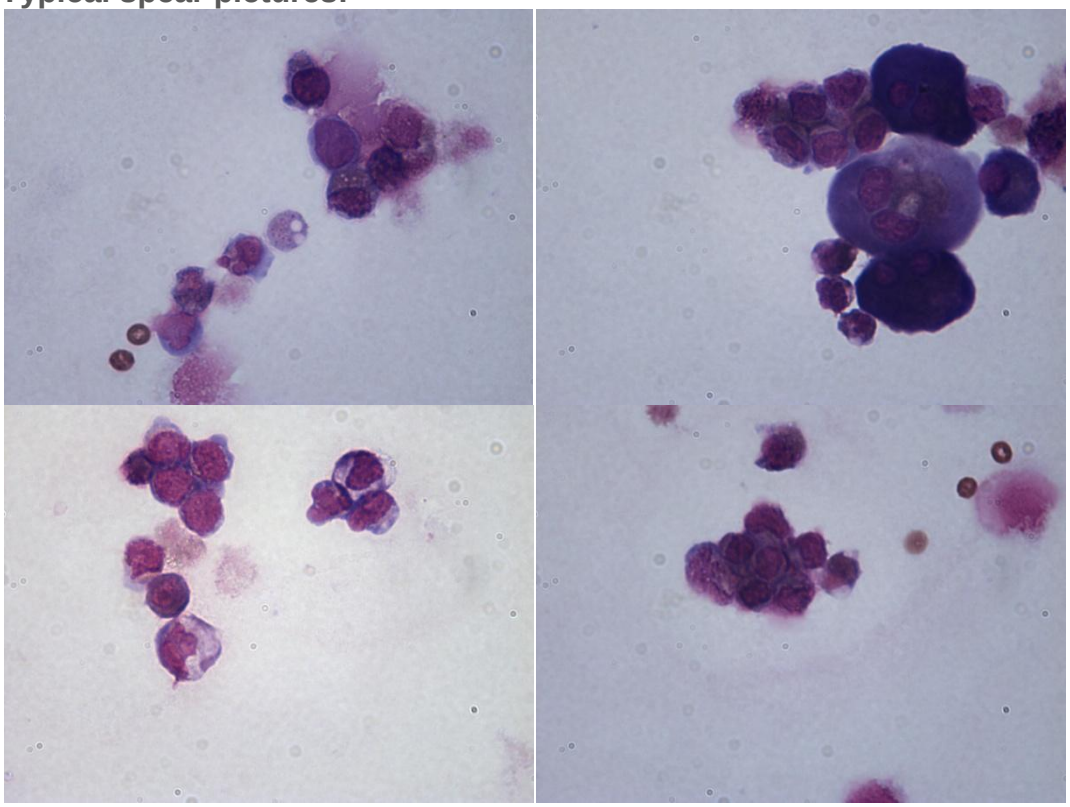
b) 30% Percoll: mix 15ml osmotic 100% Percoll with 35ml RPMI

14. Add 15ml of 30% Percoll to a 50ml Falcon tube. Underlie with 5ml osmotic 100% Percoll. To do so use a 5ml pipette together with a Pasteur pipette and another Pasteur pipette to prevent bubbles. Fill the Pasteur pipette with the 5ml pipette and let the Percoll flow down by gravity. Carefully overlay the 10ml cell suspension using the 10ml syringe. Spin the gradient at room temperature at 670 g for 30min with acceleration set to “3” and break set to “0”. (Normal acceleration 9 and break 9). Remove the cell layer at the 30/100% interphase with a 10ml pipette.



15. Put removed cells into a new 50ml Falcon tube and fill up to 45ml. Distribute the 45ml into 3x15ml Falcon tubes. Spin for 7min at 300 g.
16. Discard supernatant and pool pellets. Count yield.
17. Cells can now be used for FACS analysis, PMA/Ionomycin or peptide stimulation, etc…

Typical spear pictures:

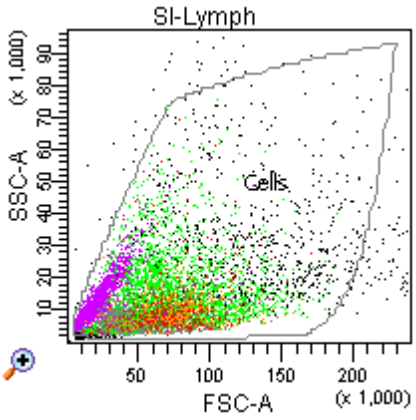


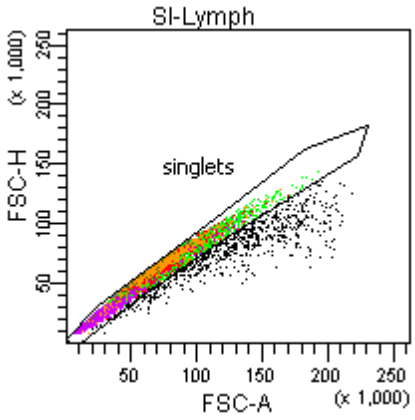
Results from FACS analysis:

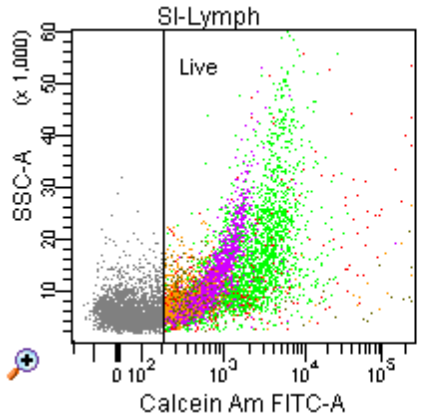
Small Intesine:

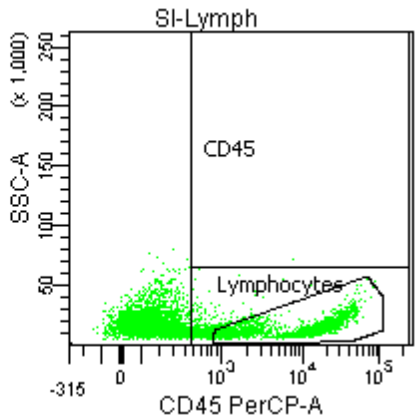
Tube: Lymph

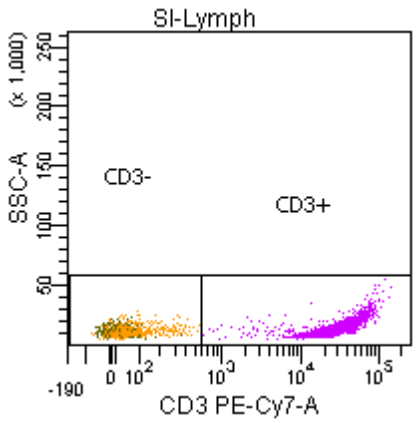
Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
Cells	9,433	94.3	94.3
singlets	8,658	91.8	86.6
Live	5,663	65.4	56.6
CD45	2,950	52.1	29.5
Lymphocytes	2,517	85.3	25.2
CD3+	1,885	74.9	18.8
CD3-	633	25.1	6.3
CD19+	356	56.2	3.6

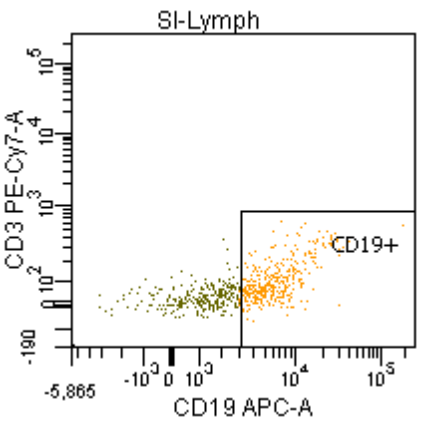












Colon:

Tube: Lymph

Population	#Events	%Parent	%Total
All Events	10,000	####	100.0
Cells	9,326	93.3	93.3
singlets	8,625	92.5	86.2
Live	7,377	85.5	73.8
CD45	6,215	84.2	62.2
Lymphocytes	5,774	92.9	57.7
CD3+	4,717	81.7	47.2
CD3-	1,057	18.3	10.6
CD19+	733	69.3	7.3