Protocol 302_Colonic Lamina Propria Lymphocyte Isolation

Reagents:

- Dissecting kit
- 1xDPBS **without $MgCl_2$ or $CaCl_2$** (GIBCO 14190-235)
- 0.5M EDTA
- 1M HEPES (Invitrogen 15630-114)
- Collagenase Type VIII (Sigma C2139-1G)
- DNAse I recombinant, Grade I (Roche 0436282001)
- Percoll (GE-Healthcare 17-0891-01)
- 10x HBSS (no sodium bicarbonate or phenol red) (Invitrogen 14065-056)
- 100µm nylon cell strainer (BD Falcon REF352360)
- 40µm nylon cell strainer (BD Falcon REF352340)
- IMDM (Gibco 12440-001)
- RPMI (Gibco…
- Pasteur Pipette (glass) 5 ¾ (VWR 14672-200)
- Gavage needle (DELVO SA, Bienne, Switzerland)
- 5ml Serological pipettes (VWR CA53300-421)
- 10ml Serological pipettes (VWR CA553300-523)
- 10ml and 20ml syringes
- Pink 18G needles
- Petridishes (VWR 25384-088)

Method:

1. Remove colon and put it in a petridish on ice containing 1xDPBS

2. (For small intestine lamina propria prep, remove peyers 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 colon and open longitudinally using a dissection scissor.

5. Put opened colon back into petridish on ice.

6. Process all mice to this stage and keep the opened colons on ice in petridish containing 1xDPBS.

7. Cut colon into 3-5mm pieces 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 37C 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).

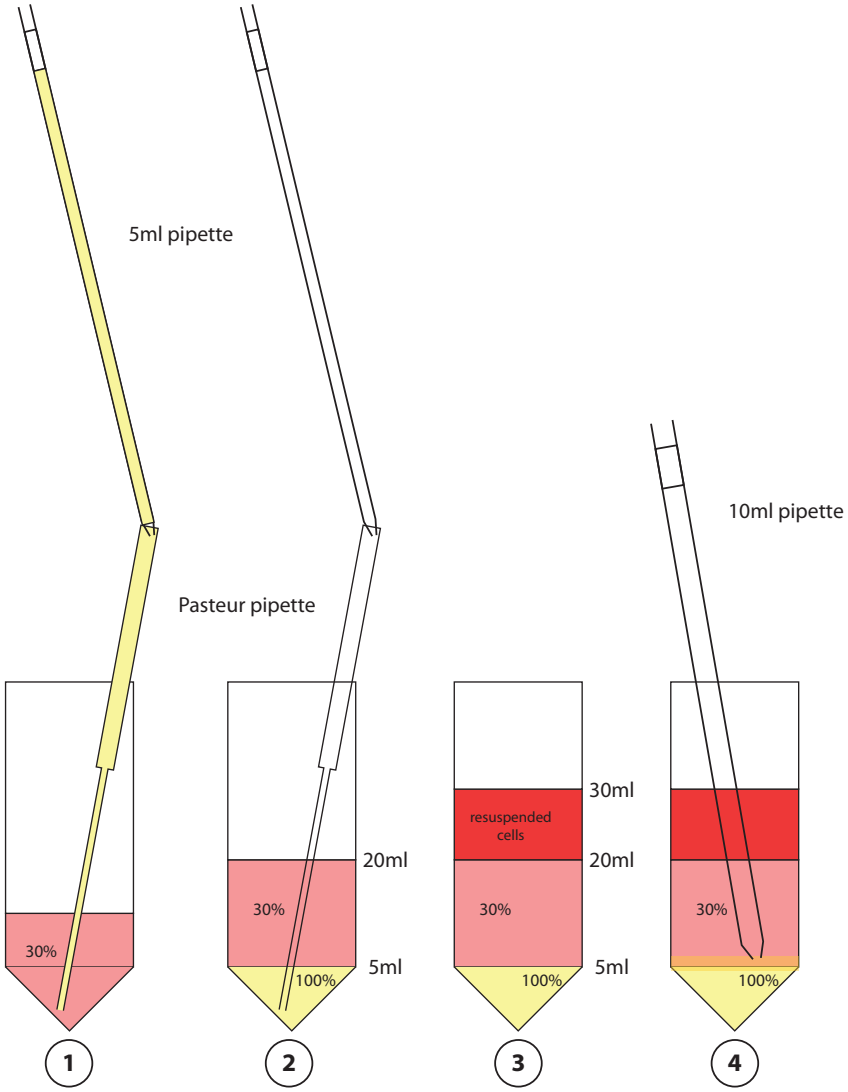
12. Disaggregate remaining tissue pieces by sucking everything through a pink 18G needle attached to a 20ml syringe. Filter everything through a 100µm nylon cell stariner into a new 50ml Falcon tube. Fill up to 50ml with IMDM. Spin for 10min at 2000rpm at 4C. Resuspend the pellet in 10ml IMDM using a 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 ③. Underly 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 ① -> ②. Carefully overlay the 10ml cell suspension using the 10ml syringe ③. Spin the gradient at room temperature at 670g 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 ④.



15. Put removed cells into a new 50ml Falcon tube and fill up to 45ml. Distribute the 45ml into 3 15ml Falcon tubes. Spin for7min at 2000rpm.

16. Discard supernatant and pool pellets. Count yield.

17. Cells can now be used for FACS analysis, PMA/Ionomycin or peptide stimulation, etc…

Notes:

Per sample individual colons can be processed. However, if higher cell yields are required 2-3 colons can be pooled per sample. If obtained cells need to be cultured for several days, fungizides need to be included, however this has not been tested yet. The prep as is will yield in contaminated long term cultures. No problems in 4-5 h PMA/Ionomycin or peptide stimulation.
Typical cell yield can reach from 10^6 - 10^7 per colon depending on the sample.