

## Isolation of lamina propria T cells

1. Sacrifice the mice and take out intestine (ileum and colon: 7-8cm)
2. Remove the feces by flushing with cold PBS/-
3. Longitudinally open the intestine and cut the pieces by 0.5-1cm
4. Add 5ml of **Solution I** to the intestine pieces (each sample in a different 50 ml tube)
5. Incubate at 37°C shaking at 250 rpm for 15 min
6. Vortex 30 sec and pass intestine through a gray-mesh (100 micron) (intestine pieces on the gray-mesh return to the original 50ml tube, collect the supernatant in 15ml tube)  
Supernatant: intraepithelial lymphocyte (IEL) + epithelial cells

Repeat steps 5-7 again (add new 5 ml of solution I to the intestine pieces and so on)

7. Add 5ml of **solution II** to intestine pieces (each mouse in a different 50 ml tube)
8. Incubate at 37°C for 45 min, shaking 250rpm
9. Vortex 30 sec and pass intestine through gray-mesh (new 15 ml tubes). This fraction contains the (lamina propria lymphocytes) LPL.
10. Centrifuge at 1500rpm for 10min at 4°C
11. Enrichment of lymphocytes (not plasma cells):  
Add 4ml of 80% Percoll to new 15ml tube  
Resuspend the pellet by 8ml of 40%Percoll  
"100%" Percoll: make 90% of Percoll (Sigma) with 10X PBS.  
90% Percoll is referred to as 100% "stock Percoll".  
From this stock Percoll, prepare 80% and 40% by diluting in DMEM 10% FBS.
12. Add the 8ml of resuspended cells to 4ml of 80% Percoll, do this step slowly and by tilting the tube in almost 180 degrees in order not to allow mix of the phases (40% and 80%).
13. Centrifuge at 2200rpm for 20min at 20°C w/o acceleration and brake.
14. Remove the supernatant by vacuum till 7ml (most of the interphase is at 5ml height)
15. Transfer the inter-phase ring to the new 15ml tube. Volume up to 15ml with PBS<sup>-/-</sup> and mix well.
16. Centrifuge it at 1400 rpm, 4°C for 8min. Remove the supernatant and stain the cells for desired markers.

### Solutions:

#### Solution I:

**HBSS + 5%FBS + 2mM EDTA + 0.15 mg/ml (1mM) DTT + 10 mM Hepes**

#### Solution II:

**5%FBS+ 1.5 mg/ml Collagenase VIII + 0.1 mg/ml Dnase I in PBS+/+**