

Mucida Lab's IEL and LPL Gut Prep

- 1) Remove intestine (remove fat and peyer's patches, fillet open, wash out feces in 3 subsequent plates with PBS). Keep WET AND COLD!
- 2) Cut into small pieces into 50mL conical with 10mL PBS
- 3) Add 10uL 1M DTT in tubes. Put tubes in holders and shake perpendicular on rolling shaker at RT speed 4.5. 10min
- 4) Intense shake 2min by hand
- 5) Filter supernatant to 50ml **IEL** conical with 10mL complete RPMI-2. Wait on ice.
- 6) Transfer tissue back to tube 1 with 10ml RPMI-2 + 600uL of EDTA (30mM final). 37C shaker 230rpm for 10min, almost horizontally. Intense shake by hand.
- 7) Filter supernatant to **IEL** tube from #5 (spin down, proceed to Percoll). **This is your IEL prep!!! (GO to PERCOLL step)**
- 8) Put tissue back into 50mL conical and add 10ml RPMI-2 + 600uL of EDTA (30mM final). 37C shaker 230rpm for 10min, almost horizontally. Intense shake by hand.
- 9) Filter out supernatant (discard).
- 10) Transfer tissue (from #8/9) back to 50mL tube with PBS and gently invert by hand for 2min. **(Alternatively you can wash the tissue directly on the metal mesh by adding PBS on top of the tissue)**
- 11) Filter out supernatant (discard). Put tissue onto 6well plate and cut for collagenase (6mL per tissue).
- 12) 45min in 37C 80rpm
- 13) Resuspend tissue several times (with 5mL pipette). Transfer to 15mL conical filtering using a nylon mash.
- 14) Spin LPL . Discard supernatant. Proceed to Percoll with Pellet (no need to filter again). **This is your LPL prep!!! (GO to PERCOLL step)**

PERCOLL step (in 15ml conical)

Resuspend in 7.5mL 40% Percoll

Underlay with 1.5mL 80% Percoll with glass syringe.

15) Spin (BRAKE OFF) 23' 2300RPM. 20C.

16) Aspirate to layer of cells. Remove blood from bottom.

17) Add media. Mix. Spin. Keep pellet.

Materials

1M DTT

500mM EDTA

Percoll:

- a. "100%" : 9x Percoll from bottle +1x PBS 10X
- b. "80%" : 80% percoll using complete RPMI and "100% Percoll"
- c. "40%" : 40% percoll using complete RPMI and "100% Percoll"

RPMI-2

500mL RPMI 1640

5mL HEPES

10mL FCS (FILTER FCS! Easier to dilute 1:1 with RPMI and filter via syringe)

Collagenase: for 50mL

49mL RPMI-2

300uL Collagenase 8 (of our 200x stock)

750uL DNaseI