

20140321 - S. Patel with adjustments (originally from A. Greer)

Lung Digestion Protocol

7mL Digestion mix in T cell media:

0.5 mg/mL Collagenase D

50 U/mL DNase I

1. Aliquot Digestion mix into a 6 well plate with cell strainers. Keep on ice.
2. Place lung onto cell strainer and mince into small chunks with scissors.
3. Place lid on the plate and incubate at 37C (in 10% CO2 TC incubator) for 45 min-1 hr
 - a. 15 mins to reach 37C and an additional 30 mins to digest
4. Shake every 10-15 minutes to dissociate clumps
5. After incubation, crush lung tissue through the cell strainer using the crested portion of a plunger from a 3mL syringe.
6. Collect media with lung cells into 15mL falcons.
7. Spin down at 1200rpm (500g) for 5 mins at 4C.
8. Red blood cell lysis: Resuspend in 1mL of TAC red blood cell lysis buffer and incubate at RT for 5 mins. Dilute to 15mL with cold PBS and spin down (500g for 5 min @ 4C)
9. Count cells.
10. Stain cells or continue with protocol as desired.

Note: Lung cells like to clump together, and can tend to clog the LSRII - vortex hard and filter through a cell strainer IMMEDIATELY before running.