Liberase/DNAase I Lung Tissue Digestion Protocol

Necessary Reagent Recipes

1. **Liberase Preparation**
   Liberase DL from Roche 277100 05401160001 (sold by sigma)
   To prepare Libersase DL: weigh out Liberase (mg) add DMEM to a concentration of 1mg/mL (stored in the -20)

2. **Bovine Pancreas DNase I Preparation**
   Bovine Pancreas Deoxyribonuclease I from Sigma-Aldrich D5025-15KU
   Type IV, lyophilized powder - 5.3mg
   Was dissolved in 2.98284 mL PBS to make 5000 Units/mL activity
   1.77683 mg/mL concentration
   56.3 microliters Bovine Pancreas DNase per mL produced 0.1mg/mL
   Concentration Calculator Link

3. **RBC Lysis Buffer**
   Prepare this fresh and discard unused. To make 2X RBC Lysis Buffer add 2mL of lysis buffer and 8mL MilliQ H2O.

4. **Wash Buffer** – 2% FBS in PBS
   Take 500mL bottle of sterile PBS Gibco pH 7.4 stored on shelf above water and bead baths
   Add 10mL of FBS (Fetal Bovine Serum) 25mL aliquots are stored at -20 in the hallway

Lung Tissue Dissociation Protocol

1. Cut up tissue in sterile petri dish or culture plate in PBS (or DMEM)
2. Place diced tissue into 50mL pre-weighed conical tube and weigh to get total tissue weight
3. Add 2mL of 1mg/mL Liberase DL (aliquots located in -20) to conical tube
4. Add DNase I to conical tube containing Liberase DL and tissue so that the final concentration is 0.1 mg/mL (for current stock this is 57uL per mL Liberase DL always double check calculations)
5. Place conical tube containing tissue and enzymes in water bath at 37 C for 35 minutes. Swirl tube regularly during digestion. Some fibrotic tissues require longer digestions if needed check every 5 additional minutes. Tissue will not appear completely in solution that is okay.
6. Remove tube from water bath when sufficient digestion has occurred. Add 5mL Wash Buffer (2% FBS PBS) to inactivate enzymes; wash through a 70 micron Miltenyi filter into a new 50mL conical tube. (set filter on top of 50mL conical and wet filter first).
   **If doing single cell on the lung use PBS with FBS only for washing (no EDTA) until the portion for single cell has been separated as EDTA interferes with emulsion reaction
7. Use the black rubber plunger of a 1 or 3mL syringe to press tissue against Miltenyi filter and rinse with more wash buffer to a final tube volume should be 40mL
8. Spin down for 10 minutes at 350xg at 4 degrees.
9. Remove supernatant and resuspend cell pellet in 2mL of PBS. Also keep this supernatant to spin down at the next step you will recover cells from this as well but they may have some RBC contamination.
10. Add 2mL of the 2X RBC lysis buffer to the resuspended cells. Mix gently once or twice then let sit for 7 minutes. Flick tube hard 5x.
11. Add wash buffer to get to desired volume for spin. Pass through a new 70uM filter into a fresh 50mL conical tube
12. Spin down for 10 minutes at 350xg at 4 degrees
13. Remove supernatant and resuspend in 2mL PBS. Pool cells from the spun down supernatant at this point.
14. Count Cells using Nexcellom Cellometer and AOPI dye. Add 20ul of cell suspension to 20uL of AOPI mix then load to disposable cell counting chamber. Use the primary cell counting program. Focus cells then count results are reported ass cells per 1 ml. Be sure to multiply final count to the volume your cell pellet was resuspended in from step 13.
15. Separate the number of cells needed for single cell if applicable and calculate number of cells to separate for each remaining assay (ie, RLT for Bulk RNAseq, Flow Cytometry, Cell Culture)