Processing of donor tissues to isolate lymphocytes

To make digestion media

- 500 ml RPMI (in cold room)
- 50ml FBS (in -20C)
- 1 tube pen/strep (in -20C)
- 5 small tubes each of (all found in -20C freeze in Joe/Kyra section)
    - DNAse
    - Collagenase
    - Trypsin inhibitor

Human Intestinal Mucosal Mononuclear/Lung Cell Isolation

1. For intestine
    a. Intestinal sample size: 4cm in length (for one 50ml tube)
    b. Gently blot mucosal surface w dry paper towels to remove excess mucus
    c. Then proceed to step 3
2. For lung:
    a. Chop piece of lung that is not lymph node and not airway. Will put 10-20ml of tissue into 50ml tube for digestion
    b. Optional: run MACS tube before digestion in addition to afterwards
3. Inject digestion media (RPMI containing 1mg/ml collagenase + 50ug/ml DNAse + trips inhibitor) submucosally
    a. Be CAREFUL when recapping syringe. Do with one hand (do not hold cap with other hand) to avoid needle stick.
4. Mince the tissue into small pieces
    a. Can also use scalpel blade here.
5. Bring total volume to 50ml w digestion media
6. Incubate for 2 hours on shaker at 37C
7. GentleMACS (C tube, lung program x2 rounds)
    a. MACS tubes are not cheap so try to reuse then.
8. Filter samples through stainless steel homogenizer tissue sieve (big mesh size screen followed by second passage through small screen)
9. **Centrifuge filtrate 5min, 400g**
10. Aspirate supernatant and resuspend pellet in RPMI. Add percoll so that final percoll percentage is 30%. Eg. Want to bring total volume to 30ml so add 9ml of percoll and then RPMI to bring to 30ml. Mix well
11. Centrifuge for 15min at 50g room temp, brakes off
12. Discard top layer and keep middle layer (transfer it to a new tube). Wash the sample with PBS/RPMI.
13. If needed, lyse red blood cells and wash.
14. Resuspend pellet into 10ml of 10% complete RPMI
15. Filter samples through 40 micron nylon filter
16. Count Cells

Human Mononuclear cell isolation from blood/bone marrow

1. Suspend in 30ml RPMI
2. Add 10ml LSM

3. Centrifuge 400g for 20 minutes brakes 1, accel 1, room temp
4. Collect lymphocyte containing middle layer
5. Centrifuge for 5 min at 150g brakes off
6. Bring total volume to 50 ml with PBS and centrifuge for 5 min at 400g at 4C
7. Resuspend pellet in 5ml AKC lysis buffer and incubate on ice for 5-10 min
8. Bring total volume to 50ml with PBS. Centrifuge for 5 min at 400g at 4C
9. Resuspend pellet in 10ml of 10% complete RPMI

Human Mononuclear Cells Isolation from lymph nodes

1. Cut tissues into small pieces ~<5mm^2. Remove fat from lymph nodes.
2. Put tissue in MACS tube and add digestion media. Incubate for 1 hour at 37 on shaker
3. GentleMACS (C tube spleen program)
4. Filter sample through small screen size sieve
5. Bring total volume to 50ml with RPMI and centrifuge 5 min 325g at 4C
6. If needed: lyse RBCs
7. Resuspend pellet in 10ml RPMI and filter through 40 micron filter
8. Count cells

Human mononuclear cell isolation from spleen
2cm^2

1. Cut tissue into small pieces, put ~20ml tissue into 50ml tube and add digestion media
2. Incubate 1hr @37C on shaker
3. GentleMACS (C tube spleen program)
4. Filter samples through stainless steel tissue sieve. First through big size screen and then second passage through small size screen
5. Put 25ml of filtrate into 50ml tube, add RPMI to bring volume to 50ml and centrifuge 5 min 325g 4C
6. Aspirate supernatant. (pellet can be large, ~10-15 ml). add 5-10 ml AKC lysis buffer and incubate on ice for 5-10 min (can be more if needed).
7. Bring total volume to 50ml with RPMI and centrifuge 5 min 325g 4C
8. Aspirate supernatant. Add percoll so that final percoll percentage is 30%. Eg. Have 15 ml pellet, want to bring total volume to 30ml so add 9ml of percoll and 6ml RPMI. Mix well
9. Centrifuge room temp 50g 12min brakes 1, accel can be fast.
10. Discard top layer (debris) and pellet (epithelial cells) and collect middle layer containing mononuclear cells.
11. Split middle layer into two tubes, bring total volume to 50ml in each tube with RPMI and spin down either a little hard or a little longer than previously.
12. Resuspend Pellet in 10 ml RPMI
13. Lyse red blood cells again if needed, and then fill to 50ml w RPMI and wash as before.
14. Filter through 40 micro filter. This step may require multiple filters. Also, to help sample go through put pipetaid onto filter and suction.
15. Count cells
16. If freezing down, then need to centrifuge cells again and resuspend in DMSO containing freezing medium*. Freeze down at approx. 10 million cells per ml.

*Freeze media is FBS with 10% DMSO. Cells should go immediately into -80 after being put into DMSO. Do not screw lid of Mr Frosties too tightly. We have color coded plastic things that pop into the lid of cryovials so that we can tell what tissues are in which vials.