**Spleen with Ammonium Chloride (150 mM)**

1. Harvest organ into 1 ml PBS-Serum, in 12-well plate on cold plate or ice

2. Move organ into 1.0 ml ammonium chloride, in 12-well plate directly on bench

3. Grind up organ with plunger of 3 ml syringe

4. Transfer through mesh into 15 ml tube.

5. Rinse empty well with 1 ml ammonium chloride and transfer through mesh

6. Bring ammonium chloride volume in 15 ml tube up to 3 ml

7. Incubate at room temperature for 10 minutes, flicking tube every 2-3 minutes to mix

8. Fill tube with PBS-Serum and spin for 10 minutes at 1400 RPM

9. Pour off supernatant, resuspend pellet in 1 ml PBS-serum

10. Fill tube with PBS-Serum and spin for 10 minutes at 1400 RPM

11. repeat one more wash

12. Pour off supernatant, resuspend pellet in 1 ml PBS-serum

**Spleen with Lympholyte**

1. Harvest organ into 1 ml PBS-Serum

2. Grind up with plunger of 3 ml syringe OR Process on GentleMACS spleen01.01 program in 2 ml PBS-Serum

3. Transfer through mesh into 15 ml tube. Rinse empty well with 1 ml PBS-Serum and transfer through mesh.

4. Bring volume in 15 ml tube up to 3.5 ml with PBS-Serum

5. Perform Lympholyte underlay with 3.5 ml, for total volume of 7 ml

6. Spin for 20 minutes at 2500 RPM, room temperature, no brake

7. Harvest lymphoid layer into new tube for staining

8. Fill to 15 ml with PBS-Serum, spin down at 1400 for 10 minutes

9. Resuspend in 1 ml PBS-Serum

**Lacrimal Glands/Submandibular Glands**

1. Harvest into 1 ml PBS-Serum

2. Place into GentleMACS tubes along with 2 ml warmed Collagenase solution

3. Heart01.01 program

4. 5 minute incubation at 37°C at 200 RPM

5. Heart01.01 program

6. 5 minute incubation at 37°C at 200 RPM

7. Heart 01.01 program

8. Transfer through mesh into 15 ml Falcon tube

9. Bring up to 15 ml with PBS-Serum

10. Spin at 1300 RPM (4 degrees) for 10 minutes

11. Pour off PBS and resuspend in 3.5 ml PBS-Serum

12. Perform Lympholyte underlay with 3.5 ml, for a total volume of 7 ml

13. Spin for 20 minutes at 2500 RPM, room temperature, no brake

14. Harvest lymphoid layer into new tube for staining

15. Fill up to 15 ml with PBS-Serum, spin down at 1400 for 10 minutes

16. Pour off PBS-Serum and resuspend in what’s left

**Liver**

1. Harvest into 1 ml PBS-Serum

2. Place into GentleMACS tube with 2 ml PBS-Serum. E.01 program on GentleMACS

3. Transfer through mesh into 15 ml Falcon tube

4. Bring up to 15 ml with PBS-Serum

5. Spin at 1300 RPM (4 degrees) for 10 minutes. Drag gently across tube rack, then resuspend in 1 ml PBS-Serum

Repeat steps 4-5 another two times (for three total washes/resuspensions)

6. Resuspend in 8 ml **40%** Percoll

7. Overlay over 3 ml **70%** Percoll

8. Spin for 20 minutes at 2500 RPM, room temperature, no brake

9. Suck off fat layer with aspirator

10. Harvest lymphoid layer into new tube for staining

11. Fill up to 15 ml with PBS-Serum, spin down at 1400 for 10 minutes

12. Pour off PBS-Serum and resuspend in what’s left

**Thymus**

1. Grind up with plunger of 3 ml syringe (like spleen) and pass through mesh into 15 ml screw-cap tube. Wash the well with 1 ml PBS-Serum and pass through mesh.

2. Fill screw-cap tube up to 15 ml with PBS-Serum and spin down at 1400 RPM, 4 degrees Celsius, for 10 minutes.

3. Pour off PBS-Serum and resuspend in remaining volume.

**Blood Staining**

1. Pipette 50 microliters heparin-sodium into a15-ml screw-cap tube. Heparin-sodium is in the refrigerator in the tissue culture room, and must be kept sterile (perform this step in the hood).

2. Extract 400-1000 microliters of blood from the mouse, using cardiac puncture, eye bleed, or tail vein bleed. Place the blood in the appropriate 15-ml tube with heparin-sodium.

3. Fill the tube with PBS-No Serum and spin at 1950 RPM, 4 degrees, for 5 minutes.

4. Dump out supernatant and re-suspend in 5 ml NH4Cl. Incubate for 10 minute at room temperature, flicking the tube every two minutes.

5. Spin down at 1350 RPM, 4 degrees, for 10 minutes.

6. Dump out supernatant, re-suspend blood pellet in 1 ml PBS-Serum, fill tube to 15 ml with PBS-Serum and spin down at 1350 RPM, 4 degrees, for 10 minutes.

7. Repeat step 6.

8. Re-suspend blood pellet in the volume of liquid remaining after dumping out the supernatant following last wash step. If the blood is clumpy, pass through mesh. Blood is ready to stain.

**Blood Serum Isolation**

1. Collect blood in 1.5 mL Eppendorf tube, without heparin

2. Keep blood on ice. Centrifuge at 14,000 rpm at 4 degrees Celsius for 20 minutes

3. Collect supernatant, being careful not to collect any red blood cells

4. Store at -20 degrees Celsius