

SOPS for General Surgery Labs:

Title: Mouse Organ Harvest

Date: 6-2012

Last Updated: 9-26-11

Solutions

- Digestion solution
 - Collagenase IV
 - Stock solution 50mg/mL (15000 U/mL depending on batch) in PBS
 - DNase I: Stock solution 20mg/mL
 - Dilute to 350U/mL collagenase and 0.02 mg/mL DNase I final concentration with RPMI+FCS, 10mL per organ
- PBS + heparin (0.1%)
- PBS + 0.1% BSA
- RPMI
- RPMI + 10% FCS
- Lympholyte M

Liver

- Place liver with 10mL digestion solution in GentleMACS C tube
 - o Run GentleMACS, program E
- Add stir bar, incubate for 40 min at 37°C, shake as much as possible
- Mesh through 70um cell strainer
- Wash with PBS-BSA
- Centrifuge at 400g for 10 min
- Wash with RPMI
- Resuspend in 5mL RPMI and overlay on 5mL lympholyte M
- Spin at 1500g for 20 min, RT, no brakes
- Take interphase, wash with PBS-BSA, centrifuge 300g for 10 min
- Resuspend and count

Lung

- Place lung with 10mL digestion solution in GentleMACS C tube
 - o Run GentleMACS, program E
- Add stir bar, incubate for 60 min at 37°C, shake as much as possible

- Mesh through 70um filter
- Wash with PBS-BSA
- RBC lysis as needed
- Wash with RPMI
- Resuspend in 5mL RPMI and overlay on 5mL lympholyte M
- Spin at 1500g for 20 min, RT, no brakes
- Take interphase, wash with PBS-BSA, centrifuge at 300g for 10 min
- Spin, decant, resuspend in PBS-BSA and count

Heart

- Place heart with 10mL digestion solution in GentleMACS C tube
 - o Run GentleMACS, program E
- Add stir bar, incubate for 60 min at 37°C
- Mesh through 70um cell strainer
- Wash with PBS-BSA
- Centrifuge at 400g for 10 min
- Wash with RPMI
- Resuspend in 5mL RPMI and overlay on 5mL lympholyte M
- Spin at 1500g for 20 min, RT, no brakes
- Take interphase, wash with PBS-BSA, centrifuge at 300g for 10 min
- Resuspend and count

Spleen

- Grind through 40um cell strainer
- Wash with PBS-BSA
- Centrifuge at 350g for 10 min
- RBC lysis with 5mL buffer for 5 min at RT
- Wash with PBS-BSA
- Spin, resuspend and count

Lymph nodes

- Grind through 40um cell strainer
- Wash with PBS-BSA
- Centrifuge at 350g for 10 min
- RBC lysis as necessary
- Resuspend and count

Peripheral blood

- Inject PB into RBC-lysis solution
 - o Incubate 5 min
 - o Quench
- Decant and resuspend in PBS in 15mL tubes
- Spin again
- Resuspend in running buffer in flow tubes
- Count

Bone Marrow

- Filter through 40um strainer
- Spin at 350g for 10 min at 4°C
- Perform RBC-lysis, 5 min, quench
- Decant and resuspend in running buffer flow tubes
- Count

Kidneys

- Could be run on GentleMACS
- Grind kidneys through 40um strainer and filter
- Wash once
- Gradient separations
 - o Optiprep:pellet:RPMI in 4:1:1 ratio
 - o Mix well
- Spin at 1450g for 20 min RT
- Take top layer, wash in 15mL running solution
- Count

