

Isakson Lab Cremasteric Vascular Isolation Procedure for 10 mice:

Premake:

**cmf-HBSS or HBSS** (*this can be made and stored in cold room*)

198 mL **cmf-HBSS** (Invitrogen cat#: 14185) or **HBSS** (14065)

2 mL pen/strep

0.119 mg HEPES (25 mM)

**Wash/Recovery Solution** in 50 mL tube

49 mL HBSS

0.5 g BSA (1%)

0.5 mL Glutamine (1%)

0.5 mL Sodium pyruvate (1%)

4.5 mg L-ascorbic acid

50 µl Fungizone (0.1%)

--On ice (separate 10 mL for recovery solution and 40 mL for wash solution)

**Beads**

- **EC-CD31 Dyna beads** (Invitrogen cat#: 111-55D)

**FBS**

20 mL

--Warmed at 37°C

**Isolation Buffer** in 15 mL tube

8.3 mL cmf-HBSS

1.3 mL DMEM

0.4 ml (200 µg) elastase (Sigma cat#: E1250; DISCARD 1 month after opening!!)

12.5 mg collagenase (Sigma cat#: C2139)

0.5 g BSA (5%)

0.1 mL Glutamine (1%)

0.1 mL Sodium pyruvate (1%)

0.9 mg L-ascorbic acid

10 µl Fungizone (0.1%)

--Warmed at 37°C

**Plating Media** in 15 mL tube

For 100 mL stock media:

1 mL glutamine (1%) (Invitrogen)

1 mL pen/strep (1%) (Invitrogen)

1 mL sodium pyruvate (1%) (Invitrogen)

20 mL FBS (20%)

77 mL MCDB-131 (InVitrogen)

*No fungizone!*

Add these components ONLY at the time of use:

--Add heparin (Sigma) 1:250 (stock solution: 10 mg/mL in HBSS) (3 days max after use)

--Add ECGS (BD Bioscience) 1:250 (stock solution: 5 mg/mL in HBSS) (3 days max after thaw)

### Autoclave:

- Forceps
- 250 mL beaker
- 80 mesh sieve

### Procedure:

- 1) Cremasters are removed and quickly placed in **recovery solution**, minimizing the opening of the lid when cremasters are put in the 50 mL tube
- 2) Once all cremaster are removed, the autoclaved forceps are used to transfer each cremaster to the **isolation buffer**
- 3) Vortex cremasters in **isolation buffer** for 60 seconds, and place in rotator at 37°C. After 8 minutes, vortex for 30 seconds. Do this a total of X 5.
- 4) Pour **isolation buffer** over 80 mesh sieve into 250 mL beaker
- 5) Gently pipette 10 ml FBS over the mesh sieve (positioned over 250 mL beaker), and another 10 mL into tube the cremasters were in, wash and recover, and pipett over the sieve
- 6) Add 10 mL of **wash medium** to the solution
- 7) Pour solution (approximately 40 mL) from beaker into 50 mL tube and spin at 1500 rpm for 5 minutes
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- 8) While solution is spinning, add 0.1 mL CD31 Dynabeads to 9.9 mL **wash solution** and gently vortex into 15 mL tube
- 9) Place the 15 mL tube into the magnet to pull beads to one side of tube for 2 minutes and remove the **wash solution**
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- 10) After the solution is done spinning, remove the supernatant and add 10 ml of **wash solution** to the remaining pellet with the cells, vortex everything well
- 11) pipett the cell slurry into the 15 mL tubes with the washed Dynabeads
- 12) vortex lightly and place on rotator at 37°C for 30 minutes
- 13) Place 15 mL tube with cell slurry and Dynabeads into magnet for 2 minutes
- 14) Remove supernatant and *gently* add 10 ml of **wash solution** to opposite side of the tube and remove from magnet and gently invert 15 ml tube 1-2 times, but *do not* vortex
- 15) Remove as completely as possible all the **wash solution** being careful not to disturb the beads
- 16) Add appropriate volume of **plating media** to the 15 ml tube and vortex to get all beads into solution
- 17) Wash with media after 24 hours