Collagenase/Dispase Mouse Brain Isolation
Yield: 3-10 x 10^7 cells/brain

Coating of Beads (do day before for best results)
1. Pipet 50ul solution of DynaBeads Sheep anti-rat IgG (Invitrogen 110-35) beads per animal into microcentrifuge tube
2. Wash 3x in 6-7ml ice cold Buffer 1 (DPBS w/o Ca & Mg, 0.1% BSA, 2mM EDTA, pH 7.4) using MPC-15 Magnet (Invitrogen 120-29)
3. Add 5ul rat anti-mouse CD31/PECAM-1 Ab (Pharmingen 557355) per 50ul beads

4. Incubate on nutator at 4C overnight or RT 30min

5. Wash 3x in Buffer 1 and resuspend in original volume w/ Buffer 1 (keep on ice)

Coating of Tissue culture Flasks
1. Dilute 2% Gelatin (Sigma G1393) in DPBS w/ Ca & Mg to 0.1%
2. Add 5ml to T-25 (one per brain) or 10ml to T-75 (one per 3-4 brains) and leave in hood for one hour
3. Remove solution and let dry in hood for a few hours

Harvest of Brain (benchtop portion using aseptic technique)
1. Use sterile technique to prepare 60mm dishes (1 for 2 brains) with 4.5ml diluted collagenase/dispase solution (0.1% dispase, 0.3% collagenase type 3 in RPMI + L-glu) and one 10cm dish with PBS
2. Sacrifice mice by CO2 asphyxiation
3. Wipe with EtOH
4. Place prone
5. Incise skin behind neck sagitally and cut rostrally, retracting skin laterally (pin down if needed)
6. Cut neck axially
7. Cut skull sagitally and pry open with thick forceps
8. Remove brain and rinse briefly in PBS
9. Place into 6cm dish w/ collagenase/dispase

Tissue Processing (tissue hood portion)
1. Mince brains in collagenase/dispase using sterile razor blade
3. Incubate 25min at 37C
4. Triturate with 5ml pipet 4x
5. Add 100ul DNAse (from 2mg/ml stock to final concentration 40ug/ml)

6. Incubate 25min at 37C
7. Transfer solution into 50ml conical
8. Rinse dish with 5ml HBSS
9. Add 2ml FBS
10. Using 10ml pipet, triturate 8-10x
11. Allow to settle
12. Remove top 5ml and strain over 100um cell strainer into new 50ml conical
13. Using 5ml pipet, triturate remaining cells 8-10x
14. Add 5ml HBSS and mix 2x
15. Allow to settle

16. Remove top 2.5ml and strain over same 100um cell strainer
17. Using 2ml pipet, triturate remaining cells 8-10x
18. Pass remainder of cells over same 100um cell strainer
19. Rinse filter with 5ml HBSS

20. Centrifuge 500g x 10min

21. Remove supernatant
22. Resuspend cells in 10.5ml RPMI solution
23. Pass over 70um cell strainer into new 50ml conical
24. Add 4.5ml Percoll dilution (9ml Percoll : 1ml 10x PBS; sterile filtered) and mix
25. Centrifuge 850g x 45min (3550 rpm on Marathon 8K centrifuge)

26. Remove all but 5ml of supernatant
27. Bring total volume to 50ml using HBSS
28. Centrifuge 400g x 10min (2400 rpm on Marathon 8K centrifuge)
29. Resuspend cells in 1ml DPBS++ and adjust cell concentration to 3 x 10^7cells/ml
30. Add 50ul PECAM1-conjugated beads per 3 x 10^7cells
31. Incubate w/ mixing 10min at RT
32. Recover bound cells/beads in magnetic separator and remove non-bound cell suspension
    a. Can save non-bound cells for further culture/separation
33. Wash cells/beads x 3 in magnetic separator using EMB-2 BASE (no serum) endothelial cell media
34. Resuspend and plate cells/beads with beads onto gelatin-coated T-25 or T-75 flasks
    a. Alternatively, to release beads immediately:
        i. Add 1ml trypsin/EDTA to cells/beads and digest 5-10min at 37C
        ii. Recover beads in magnetic separator and transfer cell suspension to new 15ml conical
        iii. Add 4ml EMB-2 COMPLETE (with serum) to inactivate trypsin
        iv. Plate cells directly onto gelatin coated plates OR centrifuge 400g x 10min, resuspend pellet and plate using the following minimum densities for plating
            - 1x10^5 cells per well in 12-well plate
            - 2x10^5 cells per well in 6-well plate
            - 1x10^5 cells per well in 6.5mm diameter Transwell Filter
        v. Also plate recovered beads in order to grow residual bound cells