Murine endothelial cell isolation

This procedure describes the isolation of endothelial cells from mouse organs including heart, lung, liver, spleen and brain using PECAM 1 antibody in immunoprecipitation.

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Day 1:
Preparation of bead for immunoprecipitation
The night before harvest: under hood using sterile technique

1. Prepare Dynabeads: use 50 µl beads per mouse harvested.

2. Wash beads in 1.7 ml Eppendorf tube by suspending beads in 0.1% BSA (diluted in 1x PBS and sterile vacuum filtered under hood), by placing tube on small magnetic separator and aspirating off supernatant. Repeat this step another 2X.

3. Suspend beads in 0.1% BSA (2 ml per 50 µl beads used) in a round bottom 15ml polystyrene tube.

4. Add anti-mouse PECAM-1; 5 µl per 50 µl beads and incubate on rocker at 4 ºC overnight.

Day 2:
Preparation of the mouse and organs:
The day of harvest

1. Euthanize mouse using CO_2 chamber.

2. Submerge mouse in 70% ethanol.

3.Working as sterilely as possible remove heart, lung, liver, spleen and brain using autoclaved instruments.

Store organs on ice in DMEM high glucose supplemented with 5ml penicillin/streptomycin.

4. Prepare Type 1 Collagenase:
Dilute 2 mg of collagenase per ml of 1% BSA.
Add 1 µl of 1M $CaCl_2$ and 1 µl of 1 M $MgCl_2$ per ml.

25 ml of collagenase is required per mouse sample.
Collagenase must be sterile vacuum filtered in hood before use.

5. Coat P100 dishes with gelatin, let sit in incubator for at least 30min, plates must be completely dried before cells are applied.

Following steps to be carried out under hood using sterile technique

1. Move organs to a Petri dish and clean fat or excess tissue.
2. Mince organs using 2 autoclaved razor blades. Do not mince longer than 1 min.
3. Transfer minced organs to a 50 ml tube containing 25 ml collagenase and let incubate in 37ºC water bath for 1 hour occasionally shaking mixture.
4. Fix a cannula to a 60 ml syringe and titrate sample 3 times.
5. Pipette mixture through a 70 µm disposable cell strainer into a fresh 50 ml tube.
6. Centrifuge 50 ml tube at 1300 rpm for 5 min at 4ºC.
7. Aspirate supernatant without disrupting pellet.
8. Re-suspend pellet in 25 ml of 0.1% BSA and again centrifuge at 1300 rpm for 5 min at 4ºC.
9. Aspirate supernatant and re-suspend pellet in 1 ml of 0.1 % BSA.
10. Wash PECAM incubated beads by placing polystyrene tube on large separator and aspirating supernatant, refill with 10-15 ml of 0.1% BSA. Repeat this step two additional times.