The Laboratory of Jason Butler, PhD
Center for Discovery and Innovation
Hackensack Meridian Health

Defined by Mike Poulos, PhD
Lab Manager – Mike Gutkin, MS
October 2020

Mouse Endothelial Cell Culture Protocols

Thawing Cells

1. Obtain cryovial from LN2 tank
2. Thaw by gently placing in 37°C water bath (do not immerse)
3. Once thawed, spray with 70% EtOH and place in hood
4. Add 9mL mEC Media to a 15 mL conical tube
5. Take thawed cell suspension from cryovial and add to 9mL mEC Media
6. Spin down for 5min at 500g (RT or 4°C)
7. Aspirate media, leaving cell pellet
8. Resuspend in 12-14mL mEC Media and place in 1 T75

Splitting Cells

1. Aspirate media from flask
2. Wash with 5mL 1X PBS for 30sec
3. Aspirate 1X PBS
4. Incubate cells with 3mL Accutase and place in 37°C until cells dissociate
5. Add 7mL mEC Media (pipette up and down to resuspend all loose cells)
6. Place all 10mL into 15 mL conical tube
7. Spin down for 5min at 500g (RT or 4°C)
8. Aspirate media, leaving cell pellet
9. Resuspend cell pellet for 1:2 / 1:3 split with final plating volume to be 12-14mL mEC media/T75

Freezing Cells

1. Aspirate media from flask
2. Wash with 5mL 1X PBS for 30sec
3. Aspirate 1X PBS
4. Incubate cells with 3mL Accutase and place in 37°C until cells dissociate
5. Add 7mL mEC Media (pipette up and down to resuspend all loose cells)
6. Place all 10mL into 15 mL conical tube
7. Spin down for 5min at 500g (RT or 4°C)
8. Aspirate media, leaving cell pellet
9. Resuspend cell pellet in 1 mL Freezing Buffer
    a. Freezing Buffer = 90% FBS and 10 % DMSO
10. Add 1mL cells in Freezing Buffer into labeled cryovial
11. Store at -80°C overnight
12. Transfer cryovials to LN2 tank