Plating Monocyte-Derived Macrophages

(APRIL 2020)

Introduction

This is the general procedure for plating macrophage cells on a 12-well flat bottom tissue culture plate. This includes counting the cells to plate them at the correct concentrations.

Definitions

PBS: Phosphate Buffered Saline
Vol: Volume
mcL: microliter

Reagents and Materials

- Monocyte Cells
  Note: These are obtained from Na Ly
  Donor ID#: _____

- Media A (see Protocol for making Media A)

- Trypan Blue (0.4%)
  Manufacturer: Fisher Scientific

- Phosphate Buffered Saline (PBS)
  Manufacturer: Fisher Scientific

- Microliter pipettor tips, sterile
  Manufacturer: Fisher Scientific

- Pipetmen
  P1000
  P20
  Manufacturer: Rainin

- Pipet tips
  P1000
  P20
  Manufacturer: Rainin

- Microcentrifuge tube rack
  Manufacturer: Fisher Scientific or USA Scientific

- Flat-bottom culture plates, 12 well, low evaporation, sterile
  Manufacturer: Fisher Scientific

Instrumentation
Cell Incubator (37 ° C, 5%, CO2 standard requirements)
Manufacturer: Fisher Scientific

Water bath
Manufacturer: VWR Inernational

Invitrogen Countess automated cell counter
Manufacturer: Invitrogen

Countess Cell Counting Chamber Slide
Lot#: _____
Manufacturer: Invitrogen

Protocol
1. Collect tube with cells from fridge in Na's lab
2. Warm Media A to 37 ℃ in water bath
3. Remove PBS from cell pellet
4. Add 1 mL warmed Media A to cells; gently pipet up and down to thoroughly mix/resuspend cells
5. Transfer suspended cells to 250 mL tube
6. Dilute cells with warmed Media A to desired cell concentration ($6.67x10^5$ cells/mL)
7. Count cells by mixing 10 mcL cell suspension with 10 mcL Trypan Blue in centrifuge tube; pipet up and down to thoroughly mix
8. Add 10 mcL mixture to cell counting chamber slide
9. Place slide in cell counter
10. Adjust focus
11. Count cells
12. Adjust cell concentration by adding more Media A as needed
13. Recount cells
14. Once at the correct cell concentration, plate cells in 12-well tissue culture plate by adding 1.5 mL of the cell/Media suspension to each well
15. Label plates; Label _____
16. Place in incubator (37 ℃, 5% CO_2)

Plate at 1 million cells/well; 1.5 mL/well; 1 million cells/1.5 mL;
0.67 million cells/mL

Final cell concentration:
Total = _____ million cells/mL
Live = _____ million cells/mL
Dead = _____ million cells/mL
Viability = _____%