Antiretroviral efficacy-Macrophages

Introduction

This protocol describes macrophage anti-retroviral efficacy of Nano formulations.

Definitions

DPBS: Dulbecco's Phosphate Buffered Saline
PFA: Paraformaldehyde

Reagents and Materials

- Plated cells in Falcon 12-well flat bottom tissue culture plate with low evaporation lid; Plate Label _____
  Manufacture: Fisher Scientific

- Drug Formulations:
  - Formulation name _____,
    Manufacture Date _____, Concentration (mg/mL) _____
  - Formulation name _____,
    Manufacture Date _____, Concentration (mg/mL) _____
  - Formulation name _____,
    Manufacture Date _____, Concentration (mg/mL) _____
  - Formulation name _____,
    Manufacture Date _____, Concentration (mg/mL) _____

- Media B
  Note: made in house via Media B protocol
  Manufacture date _____

- Cell incubator (3rd floor) Equipment ID _____; Cell incubator (8th floor, BSL2+ laboratory) Equipment ID _____

- Gibco DPBS (Dulbecco's Phosphate Buffered Saline) (1X) (without Calcium Chloride and Magnesium Chloride)
  Day 5 Lot # _____
  Day 10 Lot # _____
  Day 20 Lot # _____
  Day 30 Lot # _____
  Manufacturer: Gibco

- 10X HIV-ADA stock
  Note: obtained from Na Ly
  Passage # _____
  Date passaged _____
  Manufacturer: In House

- 4% PFA
  Label _____
  Manufacture date _____
  Manufacturer: In House

Instrumentation

Cell Incubators (37 °C, 5%, CO_2 standard requirements)
Note: *You will need to use the one in the tissue culture room and another one in the BSL 2+ laboratory for HIV infection*
Manufacturer: Fisher Scientific

Water bath
Manufacturer: VWR International

Protocol

1. Warm Media B to 37 °C in water bath (20 mL/plate(treatment))
2. Dilute formulations to appropriate concentration with warmed Media B; Treatment Concentration _____
3. Remove old Media from cells
4. Quickly, yet carefully, add 1.5 mL/well treated Media to each well
5. Place in incubator (37 °C, 5% CO_2) for 8-hour treatment
6. After 8 hours, remove treated media from cells
7. Carefully wash each well 2X with 1 mL PBS
8. Carefully add 1.5 mL/well warmed, fresh Media B to each well; cover and place in incubator until ready to infect
9. Maintain cells by doing a half media change with warmed, fresh Media B as needed (generally every other day)
10. On each infection day (D5, D10, D20, and D30 post-treatment), take plates to be infected, 9 mL Media B/plate, 4 mL Media B/plate, and 10X HIV-ADA stock up to 8th floor P3 and warm to 37 °C in water bath
11. Once warmed, dilute 10X HIV-ADA stock to 1X by adding 1 mL stock to 9 mL warmed Media B
12. Remove old media from cells
13. Quickly, yet carefully, add 1 mL/well warmed HIV Media to 9 HIV challenge wells
14. Quickly, yet carefully, add 1 mL/well warmed Media B to 3 negative control wells
15. Place in incubator (37 °C, 5% CO_2) for 16-hour infection
16. After 16 hours, remove media from cells
17. Carefully wash each well 2X with 1 mL PBS
18. Carefully add 1 mL/well warmed, fresh Media B to each well
19. Maintain cells by doing a half media change with warmed, fresh Media B as needed (generally every day)

For HIV RT activity (media from cells)

- 8 days after infection, do a full media change with warmed, fresh Media B on all wells; leave plates until day 10 (no media changes between day 8-10)
- 10 days after infection, collect media from each well into labeled centrifuge tubes for later RT assay; store at -80 ℃ (See RT assay protocol)

For p24 staining (fixed cells on plate)

- After collecting media for RT assay, carefully wash each well 2X with 1 mL PBS
- Fix cells by carefully adding 1 mL/well 4% PFA for 15 min at RT
- Remove PFA from cells and carefully wash each well 2X with 1 mL PBS for 5 min each
- Carefully add 1 mL/well PBS, wrap edges of plate with parafilm, store at 4 ℃ for later p24 staining (See p24 staining protocol)