Macrophage Cell Uptake

Introduction
This protocol describes the uptake of Nano formulations in macrophage cell cultures.

Definitions
DPBS: Dulbecco's Phosphate Buffered Saline

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) ______________
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    Manufacture Date ______________, Concentration (mg/mL) ______________
  - Formulation name ______________________________________,
    Manufacture Date ______________, Concentration (mg/mL) ______________

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

- Gibco DPBS (Dulbecco's Phosphate Buffered Saline) (1X) (- Calcium Chloride, - Magnesium Chloride)
  Lot # ___________________
  Manufacturer: Gibco

- Fisher Disposable Cell Lifter
  Manufacturer: Fisher Scientific

- Trypan Blue (0.4%)
  Manufacturer: Fisher Scientific

- HPLC grade Methanol
  Lot#: ___________________
  Manufacturer: Fisher Scientific

- Cole-Parmer Ultrasonic Processor with microtip sonication probe;
  SN ___________________
  Manufacturer: Cole-Palmer

- Round bottom culture plates, 96 well, low evaporation, sterile
  Manufacturer: Fisher Scientific

- Rubber mat, 96-well plate cover
  Manufacturer: Fisher Scientific

Instrumentation

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

Invitrogen Countess automated cell counter
Manufacturer: Invitrogen

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

Methods –

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 1, 2, 4, or 8 hour treatment time point
6. At each time point remove treated Media from cells
7. Carefully wash each well 2X with 1 mL PBS
8. Add 1 mL PBS
9. Scrape cells with disposable cell lifter
10. Collect suspended cells into labeled centrifuge tubes
11. Count cells in one representative well for each treatment by mixing 10 mcL cell suspension with 10 mcL Trypan Blue in centrifuge tube; pipet up and down to thoroughly mix
12. Add 10 mcL mixture to cell counting chamber slide
13. Place slide in cell counter
14. Adjust focus
15. Count cells
16. Spin cell suspension at 3,000 rpm for 8 minutes at 4 °C

17. After centrifugation, carefully remove and discard supernatant (being careful not to disrupt cell pellet)
18. Add 200 mcL fresh HPLC grade Methanol to each tube; At this point samples may be stored at -80 °C for later analysis or they may be processed for immediate analysis
19. If samples were stored; Remove samples from -80 °C, let warm to Room Temperature
20. Sonicate samples for 2 seconds each using the sonication probe; clean probe with 70% Ethanol between each sample, wipe dry with Kim Wipe
21. Briefly vortex each sample to ensure well mixed
22. Spin samples at 14,000 rpm for 10 minutes at 4 °C
23. Prepare standard curve for UPLC (see UPLC stds protocol)
24. Load 75 mcL of each sample into 96-well plate
25. Carefully cover plate with rubber mat; avoid any cross contamination of samples
26. Place plate in UPLC-UV to be run

Cell Count (Live; million cells/mL)				
	Treatment Time Points (hrs)			
Treatment: (formulation name)	2	4	8	12

Cell Count (Live; million cells/mL)				
	Treatment Time Points (hrs)			
Treatment: (formulation name)	16	24	Avg	Overall Avg