



# CALCEIN STAINING OF MACROPHAGES

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## BACKGROUND

- Calcein is a green fluorescent molecule whose acetomethoxy derivative (calcein AM) can be transported across cell membranes and therefore used to label live cells.
- Labelling cells with calcein AM allows for them to be quantitated using a fluorescent plate reader and is useful for various *in vitro* assays, including chemotaxis assays.

## NOTES

- If thawing out cells, first resuspend in R10 medium and allow to adhere overnight then harvest [instructions available in protocol entitled 'Harvesting Bone Marrow-Derived Macrophages'].
- If using fresh cells you will need to first harvest the macrophages from their plate [again, see 'Harvesting Bone Marrow-Derived Macrophages']
- In either case, resuspend the cells in HBSS + 0.1% BSA at 37 °C at  $1-2 \times 10^6$  cells/mL before staining

## EQUIPMENT

- Stock solutions of Pluronic F-127
  - o 20% (w/v) in DMSO, store at RT. If having trouble dissolving heat to 50 °C and vortex
  - o 50mg:250µL DMSO works well
- Stock solution of calcein AM
  - o 2.5mM in DMSO, store at -20°C
  - o 50µg:20µL DMSO works well
- HBSS + 0.1% BSA at 37°C
- Cell suspension at  $1-2 \times 10^6$  cells/mL
- 5mL polypropylene tube

## PROTOCOL

1. Prepare a 1:1 working solution of calcein AM/F-127 considering...
  - o Final concentration of F-127 in cell suspension should be <0.1%
  - o Final concentration of calcein AM should be between 1-10µM
  - o For 10µM, add 4µL of calcein and 4µL of F-127 per mL of cell suspension
2. Add working solution to cell suspension in 5mL polypropylene tube
  - o It may be useful to prepare an aliquot of unstained cells as well
3. Incubate at 37°C and 5% CO<sub>2</sub>
  - o Do not put cap on firmly as cells will not receive adequate CO<sub>2</sub>

4. To check for staining, spin cells down at 1500rpm for 5 min and resuspend in HBSS + 0.1% BSA then transfer to 96-welled black walled plate and read using fluorescent plate reader
5. Once staining is confirmed the cells are ready to use

## DISPOSAL

- Any and all media should be disposed of by diluting in 10% bleach for 30 minutes and then flushed down the sink with ample amounts of running water.