**Bone marrow-derived dendritic cell (BMDC) Isolation**

Many screening assays are performed using murine BMDCs. Below is a protocol for isolating cells for these assays.

1. Transfer bones from 1-2 x mice and media into lid of 10 cm dish

2. Fill 10 cm dish with approximately 10 mL media

3. Working one bone at a time, trim off ends

4. Take up 10 mL media with 20.5 gauge needle

5. Switch needle to tip to 27.5 gauge tip

6. Use fine tip to flush media from bones from 2 x mice

7. When flushing complete, disperse cells in media and filter bone fragments

8. Pellet cells 1,000 rpm for 5 min

9. Red blood cell lysis: Resuspend pellet in 3 mL of 0.2% NaCl for 30 sec, then dilute with 7 mL 1.2% NaCl

10. Pellet cells 1,000 rpm for 5 min

11. Resuspend in 10 mL media per bones from 1-2 mice and filter

12. Count cells

13. Plate cells at 5x10^6 cells/10 mL media/10 cm plate in DMEM + 10%FBS on petri dish (not tissue-culture treated)

14. Add recombinant GM-CSF (granulocyte-macrophage colony-stimulating factor) at final concentration of 25-50 ng/mL

15. Allow ~7d for cells to expand and differentiate