

1° Bone Marrow Dendritic Cell Isolation

Day 0:

1. Collect bone marrow from tibias and femurs from one mouse into 10ml medium (preferably RPMI with at least 5% FBS).
2. Centrifuge and decant
3. Resuspend in 5 mls ACK and incubate at 37°C for 5 min.
4. Centrifuge and decant (should have $\sim 30 \times 10^6$ cells/mouse).
5. Resuspend in 15 mls 1° DC medium/mouse and plate cells from 1 mouse/T75 flask.

1° DC Medium
RPMI+
10% FBS
L-glutamine
2-mercaptoethanol
Pen/Strep
60 U/ml GMCSF (from RDI)
10 pg/ml mIL-4 (from RDI)

6. Incubate at 37°C with 5% CO₂.

Day 2:

7. Add 15 mls 1°DC medium/flask.

Day 4:

8. Vigorously pipette medium in the flask to dislodge the DCs (the flask can be discarded).
9. Transfer medium with DC's to a 50 ml c'fuge tube (1 tube/mouse), centrifuge and decant.
10. Resuspend contents of each tube in 30 mls 1°DC medium and split between two T75 flasks.

Day 6:

Can collect immature DCs at this stage (Phenotype with anti-CD11c and B71 and B72. Should be CD-11c^{hi}.B71/B72^{lo}).

11. Gently decant medium from flask and replace with 15 ml 1°DC medium containing 20ng/ml TNF- α .

Day 8:

12. Collect mature DCs by scraping with cell scraper (phenotype with anti-CD11c and B71 and B72. Should be CD-11c^{hi}.B71/B72^{hi}).