Isolation of Erythroblasts and Megakaryocytes from Mouse Bone Marrow

1. Harvest bone marrow cells from 20-25 C57BL/6 mouse femurs and tibias (via standard flush protocol) into 10 mL PBS+5%FCS
2. Cell Count:

3. Dilute cells to 50 mL with ACK Lysing Buffer
4. Incubate 20 min on ice
5. Spin 2100 rpm (850 x g), 5 min @ 4°C
6. Resuspend in 8 mL PBS+5%FCS
7. Add 250** μL of each purified antibody: anti-

CD4	IL-7Ra	Mac-1
CD8	B220	Gr-1

8. Incubate 30 min on ice with occasional mixing

---While cells are incubating---

9. Place 8 mL magnetic beads into 8 conical tubes, mix, and apply to magnet, 5 min @ 4°C
10. Wash magnetic beads x2 with 8 mL PBS+5%FCS (add wash, vortex, apply to magnet for 5 min, and remove supernatant); leave final wash on until ready to apply cells

---Following 30 min incubation---

11. Divide cells into 2x 50 mL conicals
12. Add 45 mL PBS+5%FCS
13. Spin 2100 rpm, 5 min @ 4°C
14. Resuspend cells in each tube in 32 mL PBS+5%FCS
15. Add 8 mL cells+Ab suspension to tubes with magnetic beads and mix
16. Incubate 30 min on ice with occasional mixing
17. Mix, apply to magnet for 5 min @ 4°C
18. Remove sup to fresh 15 mL culture tube
19. Mix, apply to magnet for 5 min @ 4°C
20. Collect supernatant into fresh 50 mL conical (Lin-depleted cells)

21. Spin, 2100 rpm, 5 min @ 4°C
22. Resuspend in 8 mL PBS+5%FCS in 15 mL Falcon tube
23. Apply to slightly stronger magnet while counting
24. Cell Count:

25. Set aside 7x 5 mL FACS tubes, each with 125 μL PBS+5%FCS, labeled (single color controls):

Cells alone	PE anti-CD41	APC anti-cKit
V450 anti-CD44	FITC anti-CD61	
APC780 anti-Ter119	PerCpCy5.5 anti-Sca1	

26. Set aside 6x 5 mL FACS tubes, each with 125 μL PBS+5%FCS, labeled (multi color controls):

CD41/CD61/Sca1	cKit/CD61/Sca1	cKit/CD41/Sca1
cKit/CD41/CD61	cKit/CD41/CD61/Sca1	Ter119/CD44

27. Add 3 μL of each required antibody to the 5 mL control FACS tubes
28. Add $2x10^5$ cells into each of the 3 control-stain tubes

    ____ μL cells

29. To the remaining cells, add 150 μL of each Ab:

    ____ 150 μL FITC anti-CD44

    ____ 150 μL PE anti-Ter119

30. Incubate 30 min, on ice, with occasional mixing
31. Following incubation, add 3 mL PBS+5%FCS to each of the three control stains. Add 40 mL PBS+5%FCS to the sample cells
32. Spin 2100 rpm, 5 min @ 4°C
33. Remove supernatant and Resuspend control cells in 300 μL PBS+5%FCS and samples in 5 mL PBS+5%FCS

Sort on:

Gate: Ter119+

Sort: FSC vs. CD44+

Gate: Ter119-

Gate: Sca1-

Gate CD41+CD61+

Sort: FSC vs cKit