

Isolation of HSC and MEP from Bone Marrow cells
Bodine Lab

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Harvest bone marrow cells 20 C57BL/6 mice into 10 ml PBS+5%FCS

Cell Count:

Dilute cells in 10 volumes ACK Lysing buffer
20 minutes on ice

Spin 2100, 5 min. 4°C, resuspend in 8 ml PBS+5%FCS

Added 250 ul purified anti-CD4, -CD8, -IL7Ra, -Gr-1, -Mac-1, -Ter119, -B220.
30 minutes on ice with occasional mixing

*While cells are incubating:

Place 8 ml magnetic beads to 8 conical tubes, mix, apply 5 minutes to magnet
Wash magnetic beads 2X with 10 ml PBS + 5% FCS

Divide cells into 2 50 ml tubes

Add 40 ml PBS with 5% FCS

Spin 2100, 5 min. 4°C

Resuspend the cells in each 50 ml tube in 32 ml PBS + 5% FCS

Add 8 ml to tubes with magnetic beads, mix
30 minutes on ice with occasional mixing

Mix, place tubes on magnet for 5 minutes; 4°C
Remove supernatant (Lin- cells) into fresh tube

Mix, place tubes on magnet for 5 minutes; 4°C

Remove supernatant (Lin- cells) into fresh tube
Spin 2100, 5 min. 4°C, resuspend in 8 ml PBS+5%FCS

Cell Count:

Add ~2x10⁵ cells to 7 10 ml snap cap tubes labeled:

Cells alone	APC c-kit	PE Sca-1	AF700 CD16/32	Pac Blue CD34
c-kit, Sca-1 and CD16/32		c-kit, Sca-1 and CD34		

125 ul PBS with 5% FCS

____ ul cells

3 ul each Ab

30 minutes on ice with occasional mixing

Remaining cells:

150 ul each Ab

30 minutes on ice with occasional mixing

Add 3 ml (controls, single Abs), 42 ml (for sorting) PBS with 5% FCS

Spin 2100, 5 min. 4°C

Resuspend in 300 ul PBS + 5%FCS (controls); 5 ml (for sorting) PBS + 5%FCS for analysis

Cell Sorting:

Megakaryocyte/Erythroid Progenitors (MEP):

Lin- c-kit+/CD34-Lo CD16/32-

Post sort Count:

Hematopoietic Stem Cells (HSC):

Lin- c-kit+ Sca-1+

Post Sort Count:

Granulocyte Macrophage Progenitor (GMP)

Lin- c-kit+ CD34+ CD16/32+

Post sort Count:

Fixation:

Collect cells by centrifugation

Spin 3000, 5 min. 4°C

Resuspend in 1 ml PBS NO FCS

Add 25 ul 16% Formaldehyde (Pierce) while mixing

Room temperature 30 minutes with rotation

Quench by adding glycine to a final concentration of 125mM (.010g per tube)

Room temperature 5 minutes with rotation

Spin 3000, 5 min. 4°C

Resuspend cells in 100 ul of cold PBS, transfer to a microcentrifuge tube

Spin in microcentrifuge 3000 rpm for 5 minutes at 4°C

Freeze pellet