

Flow cytometry analysis of mouse hematopoietic stem cells

Background

Mouse hematopoietic stem cells (HSCs) are defined by the absence of lineage commitment markers, including CD45R, CD5, CD11b, Ter-119, and Gr-1, and thus are referred to as lineage-negative (Lin$^-$) cells. As they are further characterized by Sca-1 and c-kit (CD117) expression[1,2], this cell population is also termed LSK. Less than 10% of LSK cells are long-term HSCs (LT-HSCs).[3] Two main strategies are commonly used to identify mouse LT- HSCs within the LSK cell population:

1) LSK cells with no or only weak expression of CD34 and CD135[4,5]
2) LSK cells with expression of CD150 and no expression of CD48[6]

HSCs are a rare cell type in mouse bone marrow, with a frequency of about 0.002%[7]. At this low percentage, a large number of bone marrow cells would have to be acquired to achieve accurate flow cytometry analysis and effective flow sorting of HSCs. Magnetic pre-enrichment of Lin$^-$ cells however simplifies and substantially speeds up analysis and sorting of LT-HSCs.

Materials and methods

Table 1 summarizes the reagents used for enrichment of Lin$^-$ cells based on MACS® Technology and subsequent flow cytometry analysis of LT-HSCs.

Kits and reagents	Order no.
Cell separation	
Direct Lineage Cell Depletion Kit, mouse	130-110-470
Lineage Cell Depletion Kit, mouse	130-090-858
Flow cytometry	
Lineage Cell Detection Cocktail-Biotin, mouse	130-092-613
Anti-Biotin-VioBlue (clone: REA746) *	130-110-958
CD34-FITC, mouse (clone: REA383) *	130-105-831
CD135 (FLT3)-APC, mouse (clone: REA779) *	130-111-036
CD48-FITC, mouse (clone: HM48-1) *	130-102-521
CD150 (SLAM)-APC, mouse (clone: REA299) *	130-104-640
Anti-Sca1-PE-Vio 770, mouse (clone: REA422) *	130-106-220
CD117-PE, mouse (clone: 3C11) *	130-102-542

Table 1: Kits and reagents required for enrichment and flow cytometry analysis of LT-HSCs.
*For more information on additional conjugates, visit www.miltenyibiotec.com/antibodies

Enrichment of Lin$^-$ cells from mouse bone marrow

Mouse bone marrow cells were obtained by extracting the femur and tibia bones from a C57 BL/6 mouse and subsequent crushing of the bones in cold 0.5% PEB Buffer using mortar and pestle. Cells were then labeled with a cocktail of MicroBeads conjugated to antibodies against lineage markers (Direct Lineage Cell Depletion Kit, mouse). Labeled cells were depleted, leaving behind isolated unlabeled Lin$^-$ cells immediately ready for flow cytometry analysis.

Flow cytometry

Isolated Lin$^-$ cells were stained with one of two antibody cocktails (table 2) and analyzed on a MACSQuant® Analyzer 10 according to the gating strategies shown in table 3.

Cocktail 1 (for gating strategy 1)	Cocktail 2 (for gating strategy 2)
• Lineage Cell Detection Cocktail-Biotin / Anti-Biotin-VioBlue® • Anti-Sca1-PE-Vio® 770 • CD34-FITC • CD117-PE • CD135-APC	• Lineage Cell Detection Cocktail-Biotin / Anti-Biotin-VioBlue • Anti-Sca1-PE-Vio 770 • CD48-FITC • CD117-PE • CD150-APC

Table 2: Antibody cocktails enabling flow cytometry analysis of LT-HSCs according to two different gating strategies.

	Gating strategy 1	Gating strategy 2
A) All cells	Forward scatter (FSC-A) vs. side scatter (SSC-A)	
B) Viable cells	CD117-PE vs. propidium iodide	
C) Single cells	FSC-H vs. FSC-A	
D) Lin⁻ cells	Lineage Cell Detection Cocktail-Biotin / Anti-Biotin-VioBlue histogram	
E) LSK cells	Anti-Sca1-PE-Vio 770 vs. CD117-PE	
F) LT-HSCs	CD34-FITC vs. CD135-APC	CD150-APC vs. CD48-FITC

Table 3: Gating strategies for flow cytometry analysis of LT-HSCs.

Results

Lin⁻ cells from mouse bone marrow samples were isolated and analyzed by flow cytometry using gating strategy 1 (fig. 1) or 2 (fig. 2) depending on the antibody cocktail used. Although LT-HSCs represent only a small population among Lin⁻ cells, both gating strategies enabled clear LT-HSC identification.

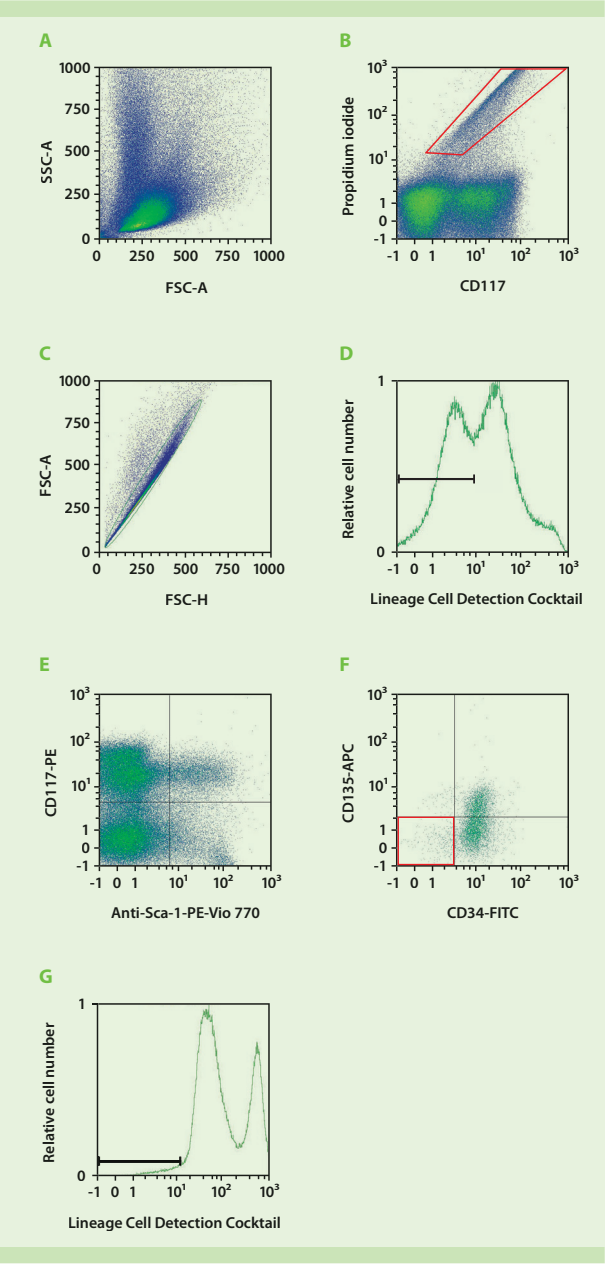


Figure 1: Identification of Lin⁻Sca-1⁺CD117⁺CD34⁻CD135⁻ HSCs. Enriched Lin⁻ cells were labeled with cocktail 1 (table 2) and analyzed by flow cytometry using gating strategy 1 (table 3). The red rectangle indicates CD34⁻CD135⁻ LT-HSCs. The gate for Lin⁻ cells (D) was set according to the original fraction (G).

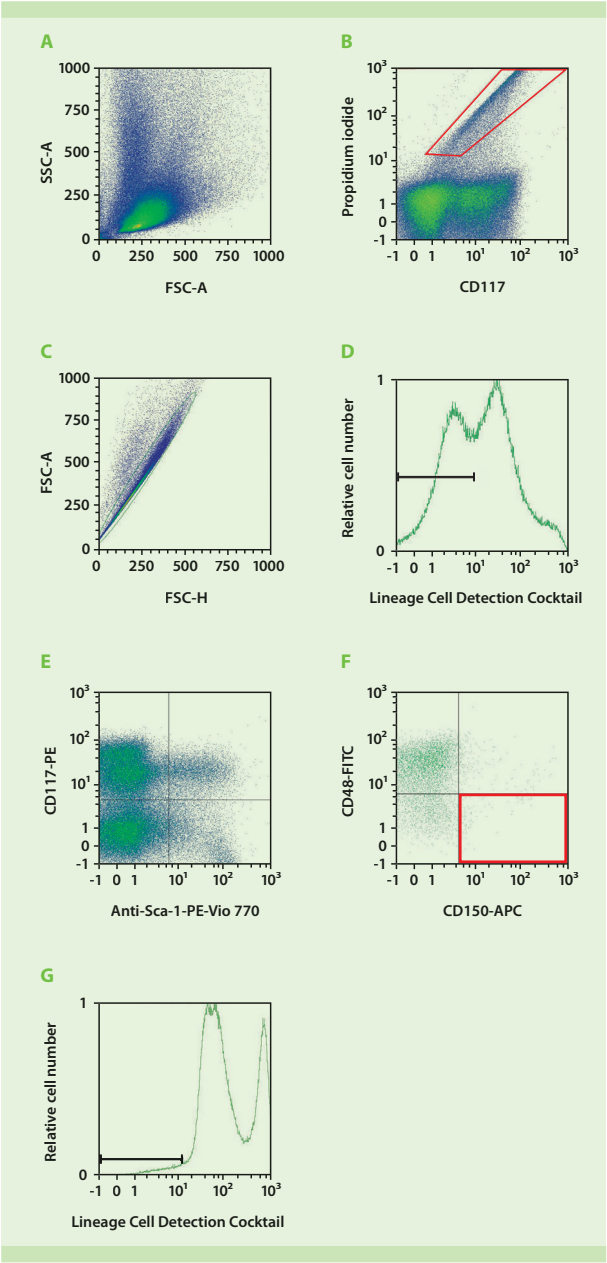


Figure 2: Identification of Lin⁻Sca-1⁺CD117⁺CD150⁺CD48⁻ HSCs. Enriched Lin⁻ cells were labeled with cocktail 2 (table 2) and analyzed by flow cytometry using gating strategy 2 (table 3). The red rectangle indicates CD48⁻CD150⁺ LT-HSCs. The gate for Lin⁻ cells (D) was set according to the original fraction (G).

Conclusion

- Multiparameter flow cytometry based on one of the two antibody panels and gating strategies enables clear analysis of LT-HSCs.
- Pre-enrichment of Lin⁻ cells by effective depletion of lineage cells from bone marrow samples simplifies and accelerates flow cytometry analysis and flow sorting of rare LT-HSCs.

References

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