

A rapid new procedure for negative enrichment of monocytes from mouse blood and bone marrow

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Introduction

Monocytes are derived from a common myeloid progenitor in the bone marrow (BM). Once released into the peripheral blood they circulate for several days before entering tissues and differentiating to macrophages. Taken together, BM progenitors, blood monocytes and tissue macrophages comprise the mononuclear phagocyte system.

Mouse monocytes are morphologically and phenotypically heterogeneous. Although their study has been facilitated by a recently developed transgenic mouse, no antigenic marker has yet been shown to be uniquely expressed by mouse monocytes. In the blood, they are known to express CD11b, F4/80 and CD115 (M-CSF receptor) but lack expression of other lineage markers (T, B, NK, dendritic cells)^{1,2}. Furthermore, monocyte subsets have been defined by their Ly-6C expression. Monocytes newly released from the bone marrow are Ly-6C^{hi} and Ly-6C expression is down-regulated in the circulation².

Methods

Preparation of a single cell suspensions

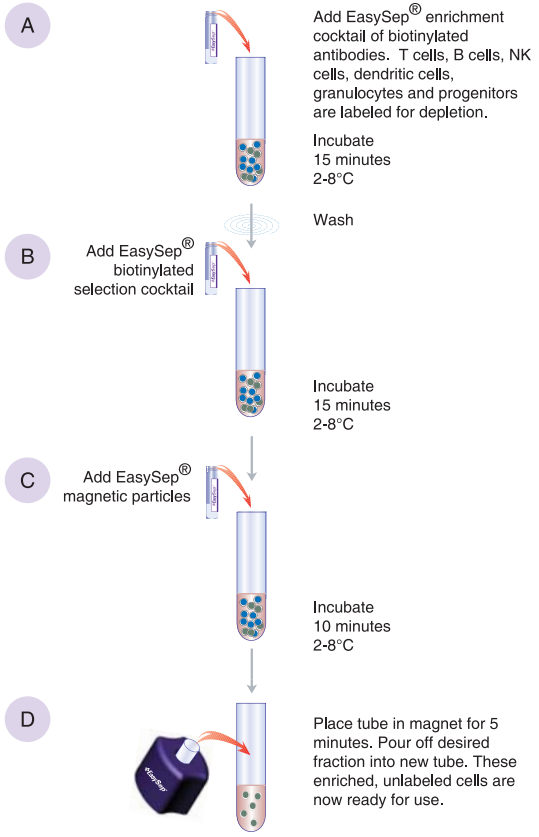
Bone marrow:
Bone marrow was harvested from 6-10 week old C57/Bl6 mice and femur and tibia bones were crushed in PBS+2% FBS and 1mM EDTA using a mortar and pestle. Clumps of cells and debris were removed by passing cell suspension through a 70 µm mesh nylon strainer. Strainer was rinsed with buffer and cells were centrifuged at 300xg for 6 minutes. Supernatant was discarded and cells resuspended at 1x10⁸ cells/mL in PBS + 2% FBS and 1mM EDTA with 5% normal rat serum added.

Blood:
Blood was collected into sodium heparin anticoagulant and lysed prior to use. Blood from 6-10 week old C57/Bl6 mice was mixed at a ratio of 1 part blood to 9 parts Ammonium Chloride (Catalog # 07800 or 07850). After incubation on ice for 15 minutes, cells were centrifuged at 300xg for 6 minutes. Supernatant was discarded and cell pellet was washed 1x with PBS + 2% FBS and 1mM EDTA. Cells were finally resuspended at 1x10⁸ cells/mL in PBS + 2% FBS and 1mM EDTA with 5% normal rat serum added. On average 4.9x10⁶ leucocytes were obtained per mL of lysed blood. Typically, 5x10⁷ BM cells or 1-2 x 10⁷ blood leucocytes were used for each protocol. The starting cell number range per experiment was 1.0 – 60.0 x 10⁷ leucocytes.

Assessing Purity:
Monocytes can express CD11b, F4/80, CD115 (M-CSFR), Gr-1 and Ly-6C. We have defined monocytes here as CD11b⁺Ly-6G⁻ using CD11b-APC and Ly-6G-PE when evaluating purity by flow cytometry.

Differentiation of enriched cells:
Enriched monocytes derived from BM were plated in MethoCult 3234 (STEMCELL Technologies) + 10ng/mL M-CSF. Cells were plated at 2000 cells/ 35mm culture dish. At d13 colonies were plucked into 100µL PBS and cytopspins made. Slides were stained with May-Grünwald Giemsa.

Figure 1. EasySep® Procedure for column-free cell enrichment



Results

Table 1. Purity and Recovery of CD11b⁺Ly-6G⁻ monocytes enriched by negative selection from bone marrow or blood using EasySep® or RoboSep®

sample	n	% purity* start	% purity enriched	Average yield of CD11b ⁺ Ly-6G ⁻ cells
Bone marrow	38	13.2 ± 2.8	88.3 ± 4.1	2.66 x 10 ⁶ per 5x10 ⁷ total start BM cells
Blood	20	14.9 ± 3.4	94.4 ± 3.0	4.08 x 10 ⁵ per 10 ⁷ total start lysed blood cells**

Values are expressed as means +/- SD
* Purities determined by flow cytometry. All samples gated on viable (PI negative) cells.
** Or approximately 2.0 x 10⁵ CD11b⁺Ly-6G⁻ cells per mL of blood based on an average of 4.9x10⁶ leucocytes per mL of blood and 1x10⁷ cells used per experiment.

Figure 2. Phenotypic characterization of mouse bone marrow or blood cells before and after enrichment (plots show viable (PI negative) cells)

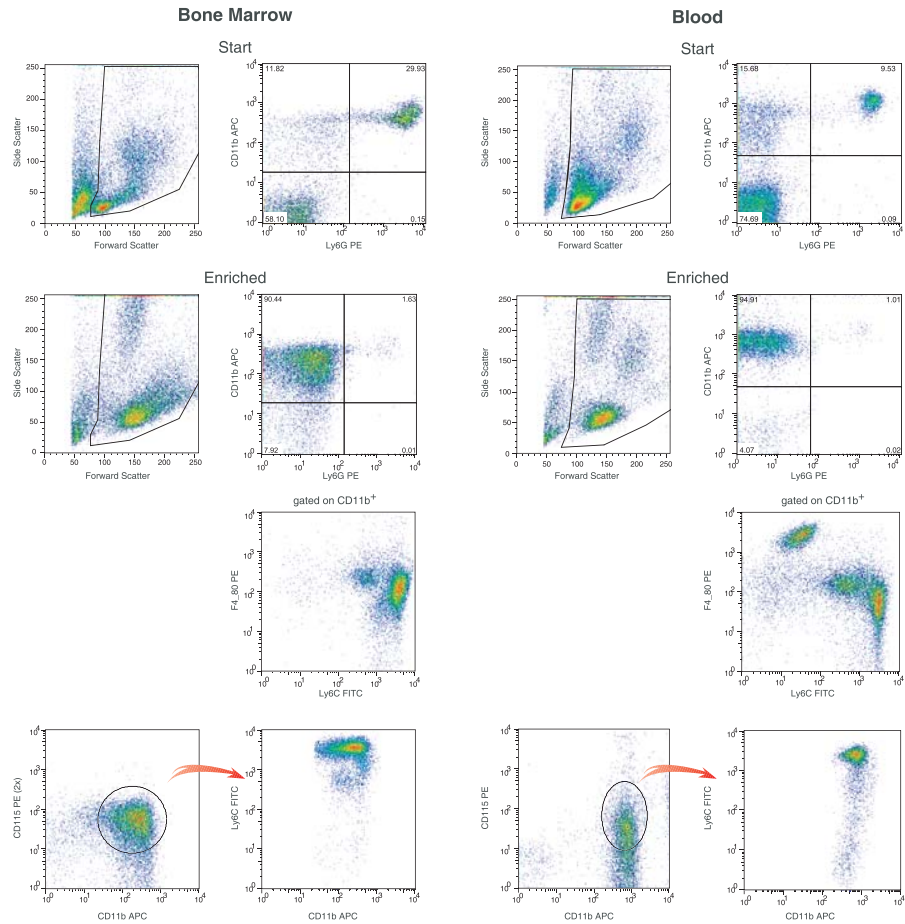
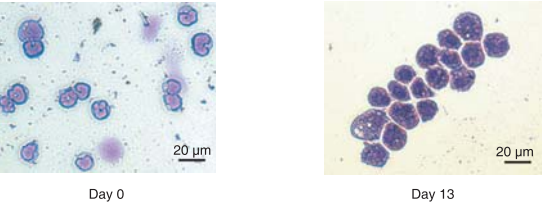


Figure 3. Cytopsin preparations stained with May-Grünwald Giemsa showing bone marrow enriched monocytes before and after 13 days of culture in MethoCult 3234 + M-CSF.



Conclusions

- No columns are required. Entire procedure takes 60 minutes
- No layering over a density medium is required to achieve high purity, viability (95%) and recovery of monocytes
- Negative selection protocol – target cells are not labeled with antibody
- Enrichment of mouse monocytes can be automated using RoboSep®
- Monocytes enriched from bone marrow and depleted of hematopoietic progenitors will differentiate to macrophages in the presence of M-CSF
- The rapidity and reproducibility of this method will facilitate the aquisition of monocytes from mouse bone marrow or blood for further study

References:
1. F. Geissmann, S. Jung, D. R. Littman, *Immunity*. 19, 71 (2003)
2. D. Strauss-Ayali, S. M. Conrad, D. M. Mosser, *J. Leukoc. Biol.* 82, 244 (2007)