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# A Simple and Fast Method for the Isolation of Untouched Mouse Pan Dendritic Cells

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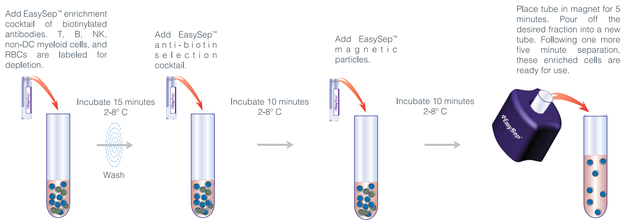
## Introduction

Dendritic cells (DC) are specialized antigen presenting cells that activate T cells while preserving self-tolerance. DC are heterogenous and various functions have been ascribed to various subsets of DC. Two major DC subsets have been described in steady-state mouse spleen: conventional DCs (cDC) and plasmocytoid DCs (pDC). Both subsets express CD11c though pDC exhibit intermediate levels of CD11c. pDC are characterized by the expression of a specific marker, PDCA-1. cDC can be further divided into two $CD8^+$ and $CD8^-$ (of which majority are $CD4^+$) subsets. Purifying DC is essential for study of their development and function but is often difficult due to the low numbers of DC. Typically, elaborate purification protocols such as FACS-based cell sorting or expansion in culture are needed to obtain enough DC for subsequent studies. Here, we describe an immunomagnetic, column-free negative selection method to isolate all DC subsets (panDC) from mouse spleen (EasySep™). Using this method, non-DC are labeled for magnetic depletion, while DC remain untouched. The whole selection is performed in approximately 50 minutes and can also be automated using RoboSep™. Upon enrichment, the panDC purities of 77 ± 9 (n=8) with a yield of 8.3 x $10^5$ cells per spleen are achieved (Table 1). Both $CD8^+$ and $CD4^+$ DC can be identified in the enriched cDC fraction. Enriched DC upregulate costimulatory and MHC class II molecules upon stimulation with LPS. In addition, they are capable of stimulating T-cell proliferation in a mixed leukocyte reaction (MLR).

## Methods

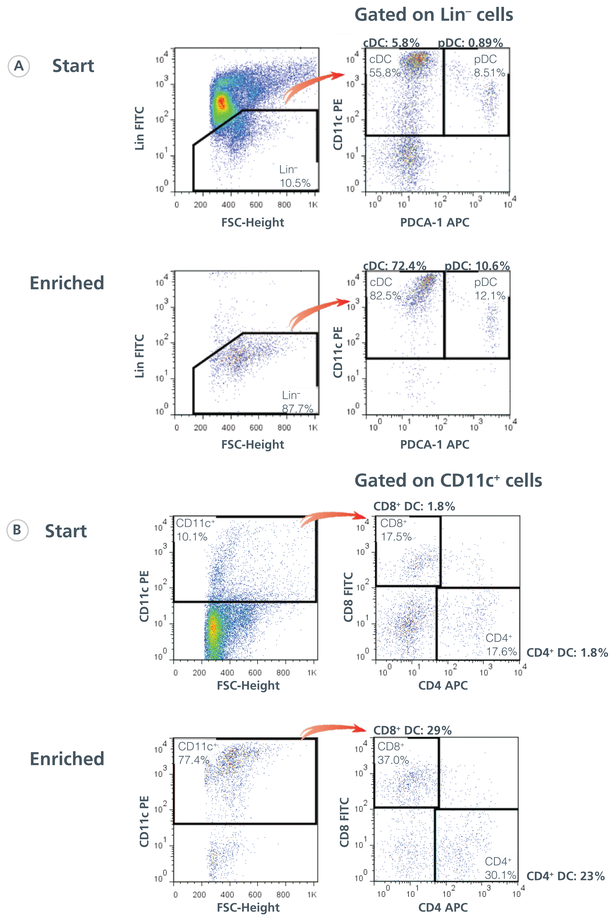
### FIGURE 1: EasySep™ procedure for column-free enrichment of panDC




The EasySep™ mouse DC negative selection kit is designed to enrich DC from mouse spleen. Briefly, spleens were digested with spleen dissociation medium containing collagenase and DNAse (STEMCELL Technologies, Catalog #07915). Single cell suspensions were prepared at a concentration of 1 x $10^8$ cells/ml in PBS + 2% FBS and 1 mM EDTA. To inhibit Fc receptor-mediated labeling, FcR blocker was added to the cell suspension before adding EasySep™ enrichment cocktail.

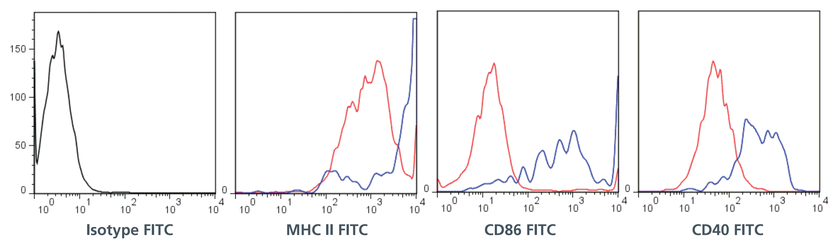
## Results

### FIGURE 2: FACS profiles before and after enrichment of DC using EasySep™




**A.** $Lin^-$ (CD3, CD19, NK1.1, Ter119, Ly-6G, F4/80) cells are gated (left panels). Conventional DC (cDC) are analysed as $Lin^-CD11c^+PDCA\text{-}1^-$, whereas plasmocytoid DC are $Lin^-CD11c^{int}PDCA\text{-}1^+$ (right panels). Total DC (cDC + pDC) purity increases from 6.7% to 83% after enrichment in this experiment. **B.** $CD11c^+CD8^+$DC as well as $CD11c^+CD8^-CD4^+$ DC are preserved in the final purified fraction (bottom panels). Frequency of target cells is calculated from total viable cells and shown outside the plots on the right.

### FIGURE 3: Expression of MHC class II and costimulatory molecules on EasySep™ enriched DC upon stimulation with LPS



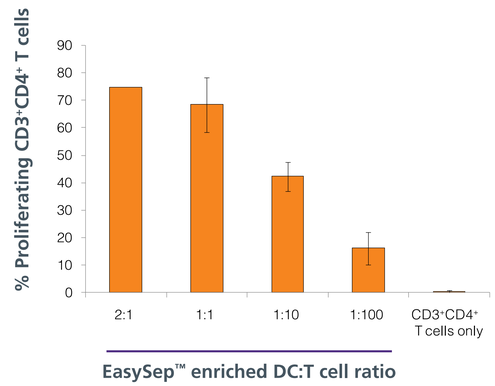

MHC class II, CD86, and CD40 expression was assessed on EasySep™ enriched DC using flow cytometry. Analysis was performed immediately after isolation (red histograms) or after overnight culture with 1 ug/ml LPS (Blue histograms). For the histogram analysis, viable $CD11c^+$ cells are gated.

### TABLE 1: Purity and cell yield of mouse panDC following negative selection using EasySep™ and RoboSep™

| | | Start | EasySep™ | | RoboSep™ | | |
|---|---|---|---|---|---|---|---|
| Cell subset | n | % Purity | Avg total cell yield/spleen | % Purity | n | Avg total cell yield/spleen | % Purity |
| cDC and pDC | 8 | 6.5 ± 1.4 | 8.3 x $10^5$ | 77 ± 9 | 5 | 6.2 x $10^5$ | 59 ± 10 |
| pDC | | 0.73 ± 0.22 | | 7.4 ± 3.2 | | | 5.8 ± 3.9 |

Values expressed as mean ± SD. Purity determined by flow cytometry. Viable cells gated using PI staining (PI negative gate) and scatter profile.

### FIGURE 4: Proliferation of allogeneic $CD4^+$ T cells in response to EasySep™ enriched DC in MLR assay




A representative of 3 experiments has been shown. $CD4^+$ T cells were enriched from Balb/C spleens using mouse $CD4^+$ T cell enrichment kit (STEMCELL Technologies, Catalog #19752) and labeled with CFSE. $10^5$ CFSE-labeled $CD4^+$ T cells were co-cultured for 4 days with different ratios of EasySep™ purified DC from C57BL/6 mice. Cell proliferation was determined by measuring CFSE dilution in viable $CD4^+CD3^+$ T cells using flow cytometry. Error bars represent standard deviation from mean of triplicate cultures.

## Conclusions

- Untouched panDC can be enriched in less than 1 hour using EasySep™ negative selection.
- Procedure can be automated with RoboSep™.
- All DC subsets are enriched and purities of 77 ± 9% can be obtained.
- EasySep™ enriched DC are not activated immediately after isolation but can upregulate activation markers upon appropriate stimulation.
- EasySep™ isolated DCs are fully functional as evident by allostimulation of $CD4^+$ T cells in MLR assays.