Isolation of Mouse CD45 Positive Leukocytes from Tissues

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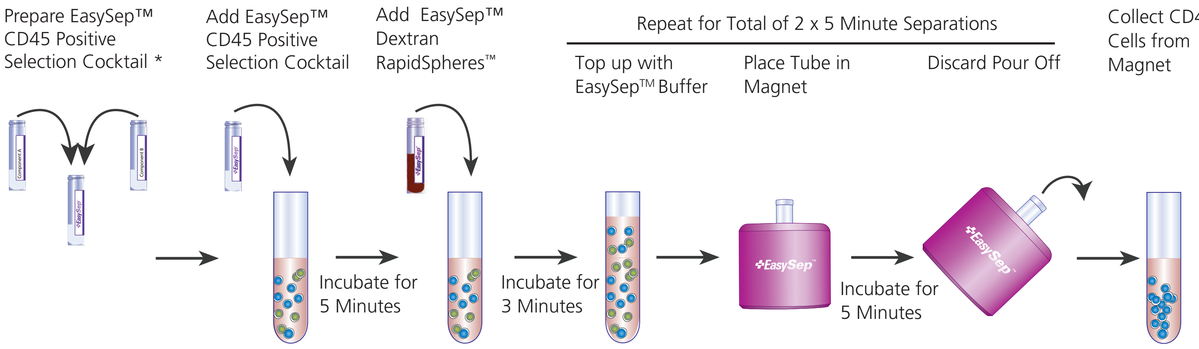
INTRODUCTION

Immune cell function is often tissue-specific, therefore, isolating cells from their tissue microenvironment is necessary to better understand their role in health and disease. Leukocyte isolation from complex tissues can be challenging in the presence of non-immune, tissue-derived cells and cellular debris from tissue dissociation. Reduced leukocyte start frequency can significantly prolong the isolation process and limit the identification of small, but critical leukocyte subset.

To overcome these obstacles, we have developed a simple and rapid immunomagnetic selection method (EasySep™) to enrich for CD45 positive leukocytes from mouse tissues.

METHODS



* Mix equal amounts of Component A and B

FIGURE 1. Easy Protocol to Isolate Mouse CD45 Positive Cells in Less Than 20 minutes

RESULTS

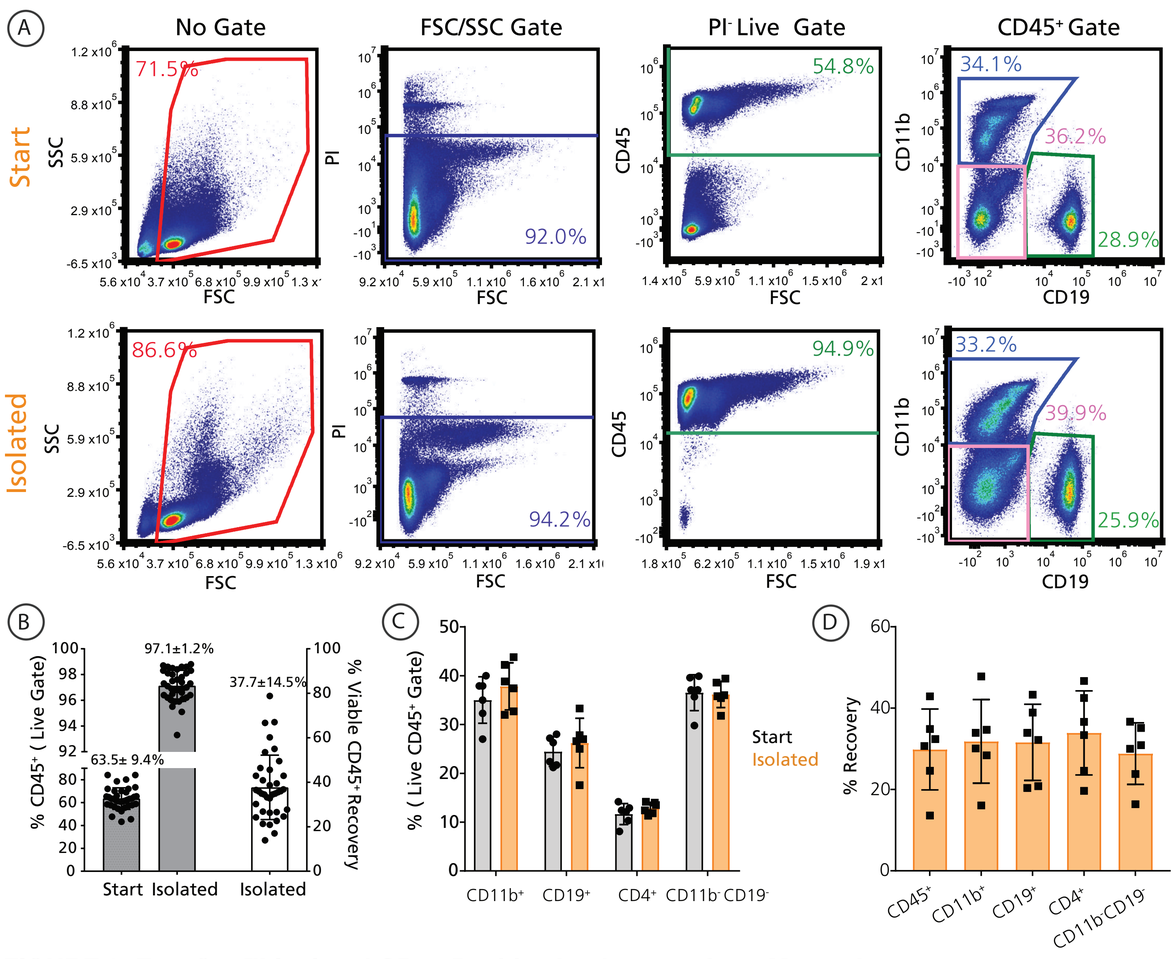


FIGURE 2. EasySep™ Isolated CD45 Positive Leukocytes from Mouse Lung

A) CD45+ cells were isolated from naive mouse lung tissues. CD45 purity (within the viable gate) and composition of immune subsets was assessed by flow cytometry. **B)** Percentage of CD45+ cells before and after EasySep™ isolation, as well as the percent recovery of viable CD45+ cell after positive selection from 37 experiments, mean +/- SD. **C)** Average frequency of immune subsets within the viable CD45+ gate and **D)** percent recovery from 6 experiments.

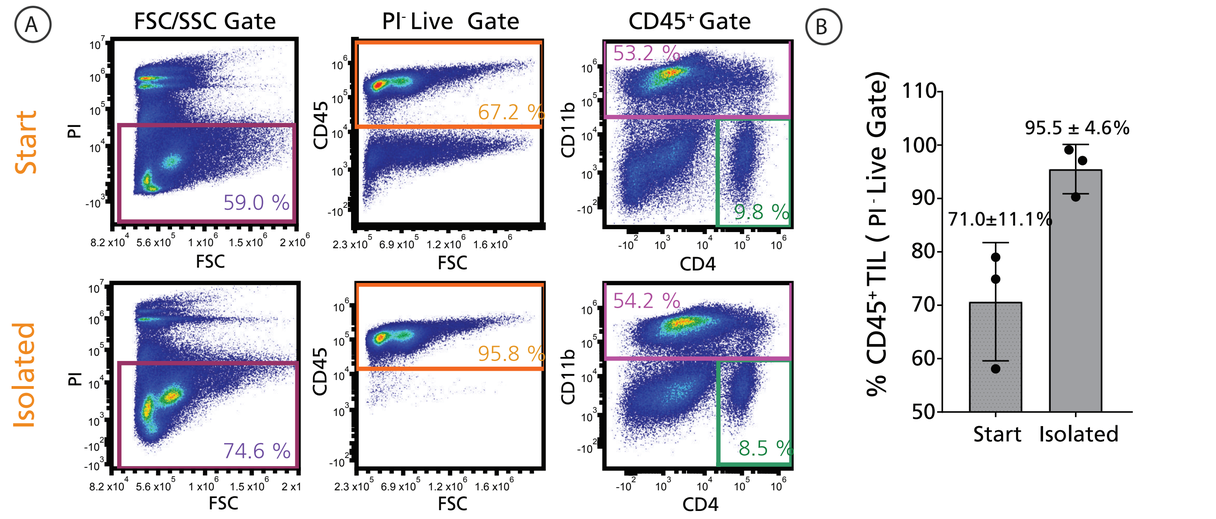


FIGURE 3. EasySep™-Isolated Tumor Infiltrating Leukocytes (TILs) from 4T1 Tumor

A) Representative flow plots of EasySep™ isolated TILs from 4T1 tumor. **B)** Average percentage of viable CD45+ TILs before and after isolation from 3 experiments, mean +/- SD.

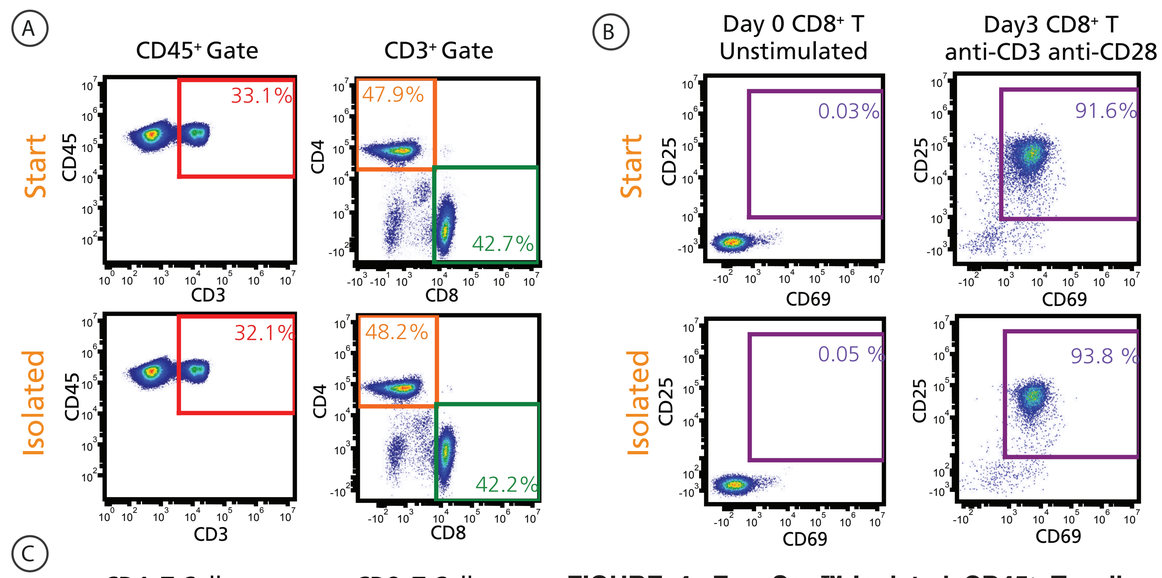


FIGURE 4. EasySep™-Isolated CD45+ T cells are Functional

A) CD45+ cells were isolated from spleens of C57Bl/6 mice using the EasySep™ Mouse CD45 Isolation Kit. Percentage of CD3+ T cells and the compostion of CD4+ and CD8+ subsets before and after isolation was analyzed by flow cytometry. **B-C)** Splenocytes were labeled with a proliferation dye then left unstimulated or stimulated with immobilized anti-CD3 and soluble anti-CD28 antibody for 3 days in ImmunoCult™-XF media. **B)** Expression of activation markers CD25 and CD69 on CD8+ T cells. **C)** CD4+ and CD8+ T cell proliferation was assessed by flow cytometry. Graph is representative of 2 experiments.

Summary

- EasySep™ Mouse CD45 Isolation Kit enriches functional leukocytes from various tissues in as little as 20 minutes.
- CD45+ cells can be isolated from lung, tumor and spleen while maintaining the relative frequency of immune subsets.
- Using healthy mouse lung tissue as an example, CD45+ leukocytes were enriched from 63.5 ± 9.4% to 97.1 ± 1.2% purity, and the recovery of viable CD45+ cells was 37.7 ± 14.5% (mean ± SD, n = 37).
- TILs were enriched from a mouse breast tumor model, 4T1 tumors from 71 ± 11.1% to 95.5 ± 4.6% (mean ± SD, n=3).
- EasySep™-isolated T cells maintain functionality as demonstrated by their ability to upregulate CD25 and CD69, and proliferate upon stimulation.