Isolation of Tumor-Infiltrating Leukocytes from Mouse Tumors

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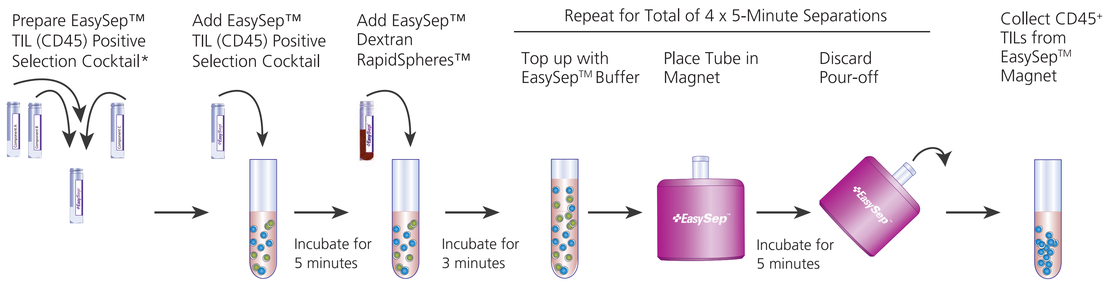
INTRODUCTION

Cell-based immunotherapy is being evaluated in various types of cancer and it is one of the most rapidly growing and promising areas of cancer research. Tumor-infiltrating leukocytes (TILs) consist of highly diverse leukocyte subsets with major roles in cancer immune surveillance. Due to their relatively low frequency, tumor heterogeneity, and the abundance of tissue debris in tumor samples, it is difficult to isolate or analyze TILs with sensitivity and precision. To address this challenge, we have developed a simple method for isolating CD45$^+$ TILs from mouse tumors. Performance was evaluated in three commonly used mouse models, namely the B16-F10 (B16) melanoma, CT26.WT (CT26) colon carcinoma, and 4T1 mammary tumor models. Solid tumors were induced by subcutaneous implantation of B16, CT26, and 4T1 cancer cell lines into syngeneic recipients. The protocol can be easily modified to achieve higher purity or recovery as required, and accomodates a wide range of sample sizes. Importantly, major immune subsets including T cells, B cells, and myeloid cells are recovered after isolation. EasySep™ Mouse TIL (CD45) Positive Selection Kit allows researchers to isolate leukocytes from tumors with ease, improving the TIL downstream workflow. Furthering our understanding of TILs will be essential for developing effective immunotherapeutic strategies.

METHODS



*Mix Component A, B, and C at a 1:1:6 ratio

FIGURE 1. EasySep™ Mouse TIL (CD45) Positive Selection Protocol

RESULTS

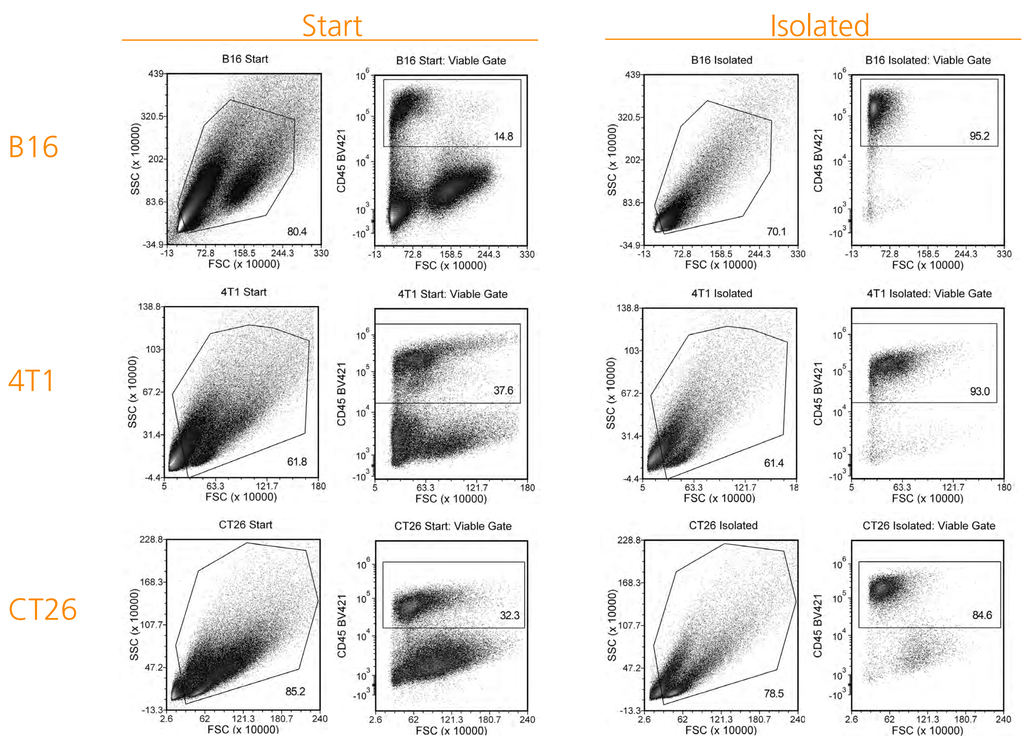


FIGURE 2. CD45$^+$ TIL Purity Before and After EasySep™ Isolation. CD45$^+$ TILs were isolated from single-cell suspensions at various start concentrations using the EasySep™ (purple) Magnet. B16, 4T1, and CT26 samples were adjusted to 1 x 10^8 cells/mL, 4 x 10^7 cells/mL, and 2.5 x 10^7 cells/mL, respectively prior to EasySep™ isolation. CD45$^+$ TIL purity within the viable cell population (PI negative) was assessed by flow cytometry.

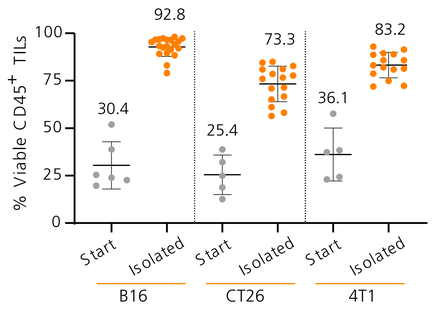


FIGURE 3. Performance of EasySep™ Mouse TIL (CD45) Positive Selection Kit in Three Tumor models. TIL isolation from B16, CT26, and 4T1 samples starting at 1 - 10 x 10^7 cells/mL, using the EasySep™ (purple) Magnet. Data shown as mean purity +/- SD.

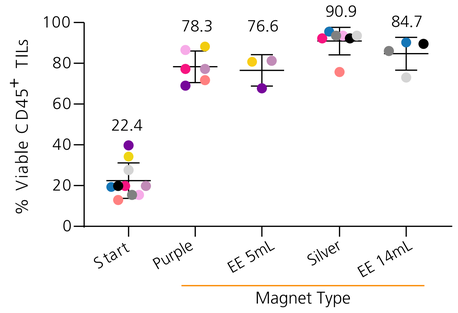


FIGURE 4. Compatibility of CD45 Positive Selection Protocol with Various EasySep™ Magnets. TIL isolation from B16 tumor samples using the EasySep™ (purple), Big Easy™ (silver), and EasyEights™ (EE) Magnets. Samples are prepared at 1 - 2.5 x 10^7 cells/mL, 0.5 - 1 mL. Data shown as mean purity +/- SD. Each color represents an independent experiment.

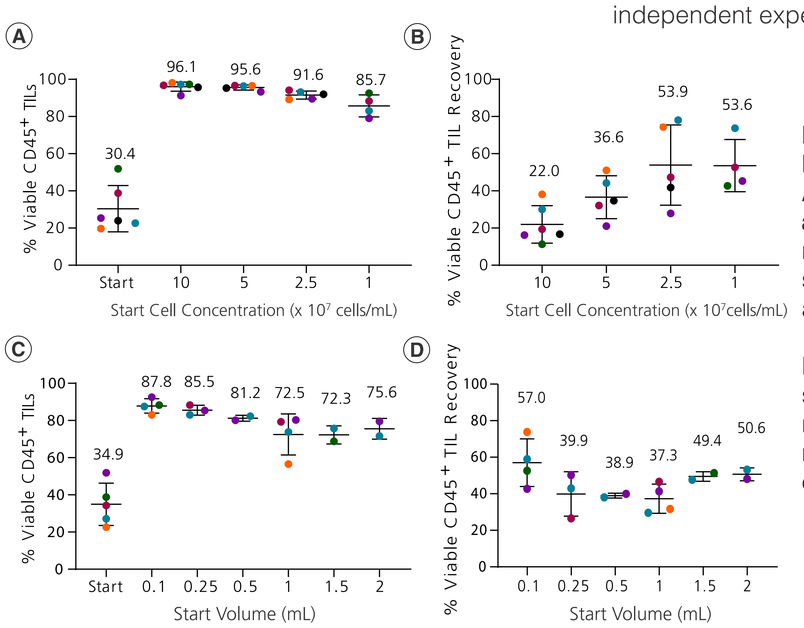


FIGURE 5. Higher TIL Purity or Recovery can be Achieved by Adjusting the Start Concentration and Volume. CD45$^+$ TIL purity and recovery in B16 samples at various start cell concentrations **(A,B)**, and across different start volumes at 1 x 10^7 cells/mL **(C,D)** using the EasySep™ (purple) Magnet. Data shown as mean purity or recovery +/- SD. Each color represents an independent experiment.

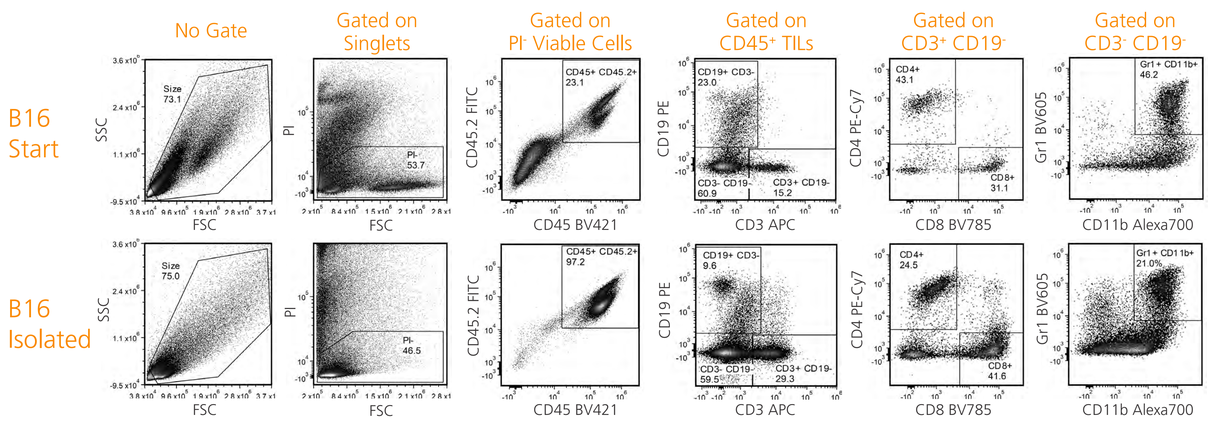


FIGURE 6. Immune Cell Composition of a B16 Tumor Before and After EasySep™ TIL (CD45) Positive Selection.

Summary

- EasySep™ Mouse TIL (CD45) Positive Selection Kit enables the isolation of leukocytes from solid tumors in as little as 30 minutes, while achieving purity as high as 98%.
- The isolation protocol can accommodate different sample volumes and cell densities due to tumor tissue heterogeneity.
- TIL purity or recovery can be optimized by adjusting the starting cell concentration or volume.
- TIL immune cell composition of the starting sample is maintained following EasySep™ isolation.