Isolation of Human CD45+ Leukocytes From Tissues and Human Tumor Xenografts in Humanized Mice

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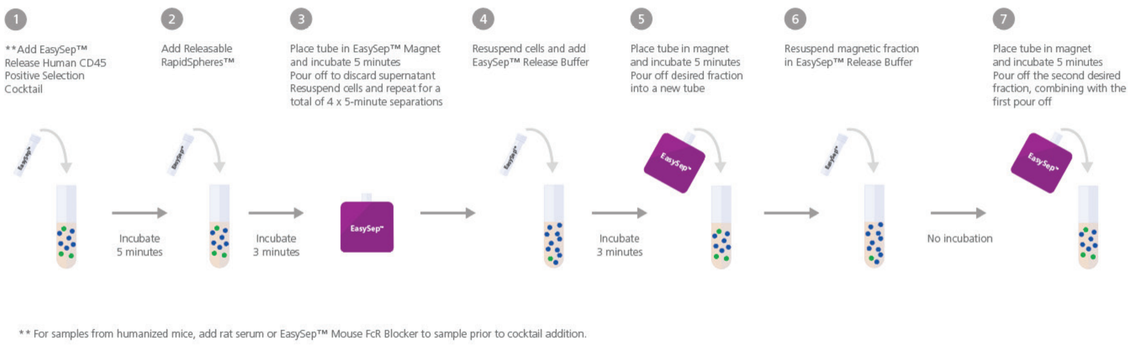
INTRODUCTION

The study of immune cell function in non-lymphoid tissue and tumors has emerged as an exciting research area with the promise to provide novel strategies for the treatment of immune disorders, infectious diseases, and cancer. A major challenge in the field is the isolation of leukocytes from tissues and tumors, due to their complex structure and variable composition. We describe here a new protocol for the isolation of particle-free, human CD45+ leukocytes from dissociated tissues or tumors. Using the EasySep™ Release Human CD45 Positive Selection Kit, leukocytes are labeled with antibody complexes linked to magnetic particles and separated using an EasySep™ magnet. The isolated cells are then released from the magnetic particles by resuspension in EasySep™ Release Buffer and a final magnetic separation step.

Cell separation performance was assessed using a humanized mouse tumor model whereby NRG-3GS mice were engrafted with human CD34+ hematopoietic stem and progenitor cells and xenotransplanted with human breast (MDA-MB-231) or ovarian (SKOV3) cancer cell lines. In isolations from humanized mouse tissues, the starting and isolated human CD45+ frequency ranges were 6.0 - 57.2% and 90.9 - 99.4%, respectively (n = 3). Starting with human tumor xenografts, tumor-infiltrating leukocytes (TILs) were enriched from a starting range of 0.4 - 18.0% to 76.6 - 92.7% following isolation (n = 4). The resulting immune cell subset frequency distribution is representative of the starting population, and further separation of immune subsets can be achieved with additional downstream isolation.

Humanized mouse models of clinical disease are instrumental in furthering our understanding of complex mechanisms of disease progression or resolution. This new kit for the isolation of human immune cells from tissues and tumors will facilitate further examination of the roles of immunity in disease and the evaluation of immune-based treatment strategies.

METHODS



** For samples from humanized mice, add rat serum or EasySep™ Mouse FcR Blocker to sample prior to cocktail addition.

FIGURE 1. EasySep™ Release Protocol for Isolation of Particle-Free Human CD45+ Leukocytes in Less Than 45 Minutes

Prior to separation, human peripheral blood mononuclear cells (PBMCs) were spiked into single-cell suspensions of mouse thymocytes at a PBMC frequency range of 1 - 30%. For isolation of human leukocytes from humanized mouse tissues, single-cell suspensions were prepared from spleen, dissociated lungs, and dissociated tumors. Cells were resuspended at a concentration of 1 x 10^8 cells/mL in phosphate-buffered saline (PBS) containing 2% FBS and 1 mM EDTA.

RESULTS

TABLE 1. EasySep™ Release Human CD45 Positive Selection Kit Performance From Human PBMCs Spiked into a Single-Cell Suspension of Mouse Thymocytes Across a Range of Frequencies

To simulate the variable leukocyte content in tissue and tumor samples, PBMCs were spiked into a single-cell suspension of mouse thymocytes at a frequency of 1% or 10 - 30%, and EasySep™ isolation was performed. Start frequencies and isolated sample purities (percentage of human CD45+ cells within the viable cell gate) were assessed by flow cytometry.

	Start Frequency 0.7 - 2.2% CD45+	Start Frequency 8.1 - 36.7% CD45+
% Purity (mean ± SD)	96.9 ± 1.5	99.6 ± 0.3
% Recovery (mean ± SD)	63.1 ± 19.6	70.5 ± 9.9
n	11	11

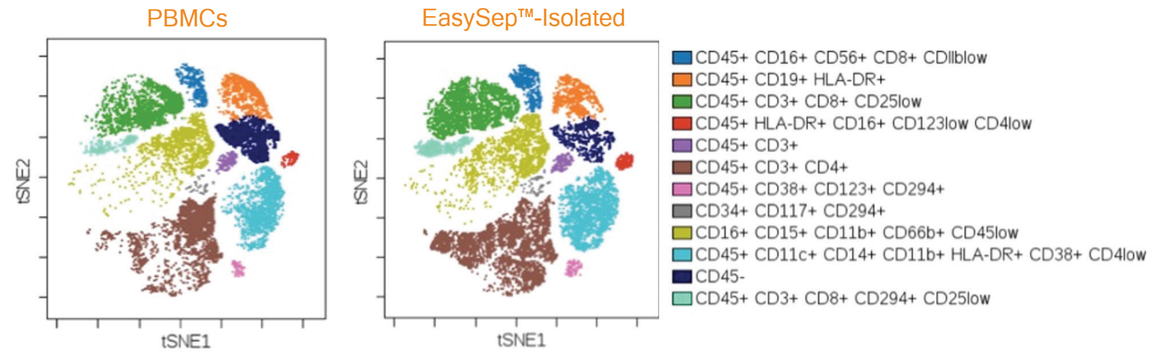


FIGURE 2. EasySep™-Isolated CD45+ Cells are Representative of the Starting Leukocyte Population

Mass cytometry data comparing the composition of immune subsets in human PBMCs and EasySep™-isolated cells from the same donor. Starting with whole blood, PBMCs were prepared by density gradient centrifugation using Lymphoprep™. To compare immune subset composition pre- and post-EasySep™ isolation, a fraction of the PBMCs was further isolated using EasySep™ Release Human CD45 Positive Selection Kit, and the pre- and post-isolated fractions were assessed by mass cytometry (CyTOF®). t-SNE plots of cells stained with 19 markers and analyzed by CyTOF® are shown (n = 1).

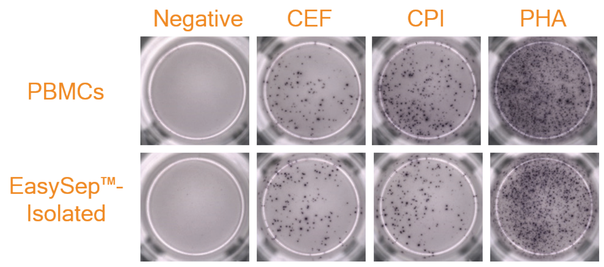


FIGURE 3. EasySep™-Isolated CD45+ Cells Produce IFN-gamma in Response to Antigen and Mitogen Stimulation

Human PBMCs and EasySep™-isolated cells were incubated for 24 hours in the presence of peptide pools (CEF for antigen-specific CD8+ T cell response and CPI for antigen-specific CD4+ T cell response) or mitogen (phytohemagglutinin [PHA]). Following incubation, ELISpot plates were processed and IFN-gamma-producing cells were counted using an AID ELISpot reader. Representative images of ELISpot assays are shown (n = 3).

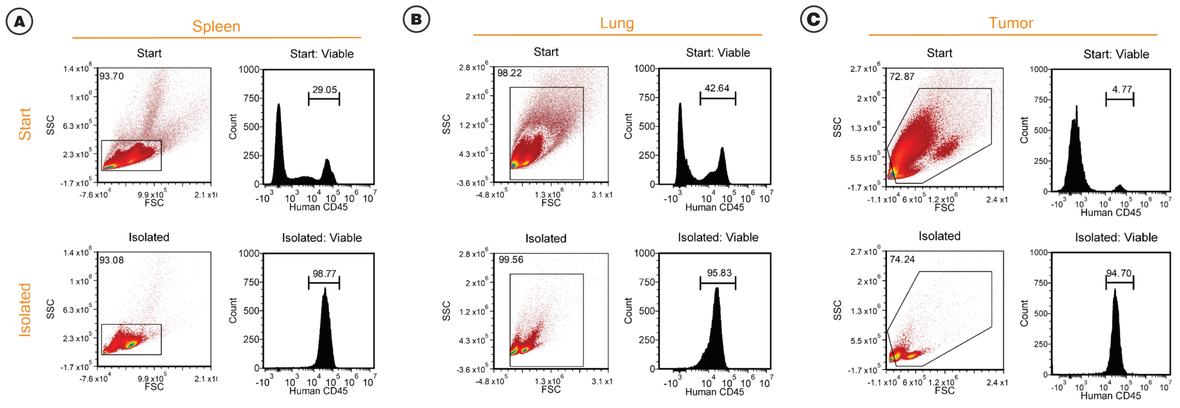


FIGURE 4. Isolation of Human Leukocytes From Tissues and TILs from Human Tumor Xenografts of Human CD34+ Cell-Engrafted NSG™ Mice Using EasySep™ Release Human CD45 Positive Selection Kit

Human CD45+ cells were isolated from (A) spleen, (B) dissociated lungs, and (C) dissociated MDA-MB-231 tumors from human CD34+ hematopoietic stem cell-engrafted NSG™ mice. CD45+ purity (within the viable cell gate) was assessed by flow cytometry. Representative plots (n = 3) for each tissue type are shown.

Summary

- EasySep™ Release Human CD45 Isolation Kit isolates leukocytes from various tissues and start frequencies in less than 45 minutes
- Using human PBMCs spiked into mouse thymocytes as a model, isolated human CD45+ purities were 96.9 ± 1.5% from start ranges of 0.7 - 2.2% (n = 11) and 99.6 ± 0.3% from start frequencies of ≥ 8% (n = 11)
- Isolated human immune cell subsets are representative of the starting leukocyte population and maintain functionality as demonstrated by IFN-gamma ELISpot assays
- Isolation of human leukocytes from humanized mouse tissues with start frequencies of 6.0 - 57.2% resulted in purities ranging from 90.9 - 99.4% (n = 3)
- Starting with human tumor xenografts, TILs were enriched from a starting range of 0.4 - 18.0% to 76.6 - 92.7% following isolation (n = 4)