Automated Large-Scale T Cell Isolation in a New Closed Cell Separation System

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INTRODUCTION

Large-scale T cell isolation is commonly performed in a wide range of laboratory settings, including for cell therapy and drug discovery research, as well as in core facilities as part of cell manufacturing and banking workflows. However, current methods can be a significant bottleneck in a lab's workflow, often requiring a full day just for sample processing and cell isolation. Furthermore, many applications have stringent requirements for purity, sterility, and standardization of the cell isolation procedure. To address these needs, we have developed RoboSep™-C, an instrument for efficient and automated cell isolation in a closed system.

RoboSep™-C automates the established EasySep™ technology for immunomagnetic cell separation to enable isolation of untouched T cells, $CD4^+$ T cells, or $CD8^+$ T cells from leukapheresis samples. To set up the system, the user follows the on-screen prompts to install a sterile single-use tubing set and load the recommended medium, cell isolation reagents, and starting Leukopak. The instrument then performs all necessary cell processing steps including sample washing, cell labeling, magnetic separation, and cell concentration in a 50-minute protocol.

RoboSep™-C-automated isolation yields functional T cells with high purity, recovery, and viability. This new platform enables researchers to scale up their operations and can be easily integrated upstream of existing T cell expansion, genome editing, and cryopreservation protocols.

METHODS

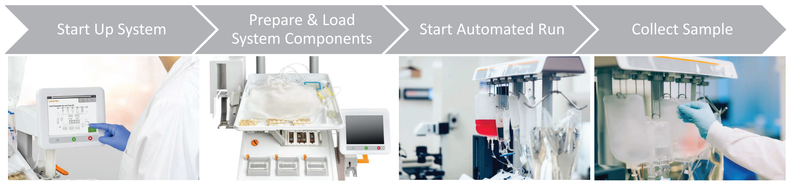

FIGURE 1. RoboSep™-C Cell Isolation Protocol: Overview of Instrument Setup and Automated Protocol for Isolation of T Cells, $CD4^+$ T Cells, or $CD8^+$ T Cells

To initiate a run, all system components—including the RoboSep™-C Tubing Set, Buffer, and Cell Isolation reagents and the starting Leukopak (2.5 to 20 x 10^9 nucleated cells)—are loaded onto the instrument. RoboSep™-C automates the cell washing and isolation protocol within the closed, sterile single-use tubing set. Cell concentration, buffer exchange, and platelet removal are performed in the Cell Wash Cartridge of the tubing set. Non-T cells, including red blood cells (RBCs) and platelets, are targeted for depletion by the RoboSep™-C T Cell Isolation reagents. Estimated total protocol time is 75 minutes (25 minutes hands-on time and 50 minutes for the automated run).

RESULTS

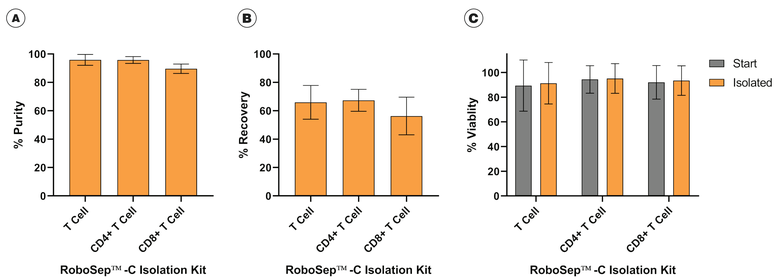

FIGURE 2. RoboSep™-C Enables Large-Scale Automated Isolation of Human T Cells with High Purity, Recovery, and Viability

(A) T cells, $CD4^+$ T cells, or $CD8^+$ T cells were isolated from fresh human peripheral blood Leukopaks (2.5 to 20 x 10^9 start cells) using RoboSep™-C. Purities of T cells ($CD3^+$), $CD4^+$ T cells ($CD4^+CD3^+$), or $CD8^+$ T cells ($CD8^+CD3^+$) within the viable $CD45^+$ population was assessed by flow cytometry. **(B)** Recoveries are shown as the number of target cells in the isolated fraction divided by the number of target cells in the start sample x 100%. **(C)** Pre- and post-isolation viability was assessed by staining with the cell viability dye DRAQ7™ and analyzed by flow cytometry. Data are shown as mean ± SD; n=16 - 20.

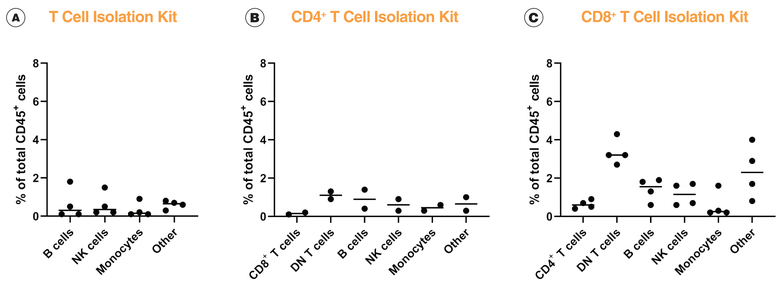

FIGURE 3. RoboSep™-C-Isolated Cell Populations are Highly Pure with Minimal Contaminating $CD45^+$ Cells

T cells, $CD4^+$ T cells, or $CD8^+$ T cells were isolated from fresh human peripheral blood Leukopaks (between 2.5 and 20 x 10^9 start cells) using RoboSep™-C. The contaminating nucleated cell frequencies in the final fractions, isolated using the **(A)** T Cell, **(B)** $CD4^+$ T Cell, or **(C)** $CD8^+$ T Cell Isolation Kits, were assessed by flow cytometry. Cells were identified by cell surface marker staining: B cells ($CD19^+$), NK cells ($CD56^+CD3^-$), monocytes ($CD14^+$), $CD4^+$ T cells ($CD4^+CD3^+$), $CD8^+$ T cells ($CD8^+CD3^+$), double-negative (DN) T cells ($CD4^-CD8^-CD3^+$), and other ($CD45^+$ cells not identified by the staining panel). Graphs show individual data points and the mean; n=2 - 4.

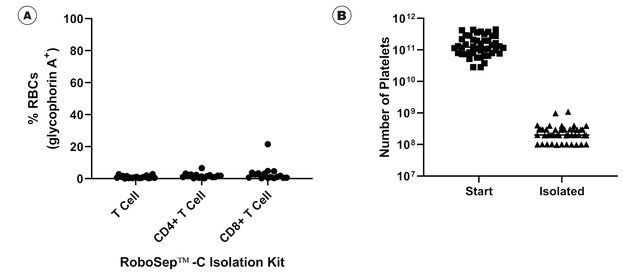

FIGURE 4. RoboSep™-C is Highly Effective at Removing RBCs and Platelets from Leukopak Samples

T cells, $CD4^+$ T cells, and $CD8^+$ T cells were isolated from fresh human peripheral blood Leukopaks (2.5 to 20 x 10^9 start cells) using RoboSep™-C. **(A)** RBC frequency (% glycophorin A^+) in the isolated sample was assessed by flow cytometry. RBC to white blood cell ratios in the starting sample were typically > 2:1. **(B)** The number of platelets in the start and isolated fractions (T Cell, $CD4^+$ T Cell, and $CD8^+$ T Cell Kits combined) was determined using a Coulter Hematology Analyzer. Graphs show individual data points and the mean; **(A)** n=16 - 20 and **(B)** n=46.

(A)

	Start	Isolated
T Cell % ($CD3^+$)	55.2	95.0
CD4:CD8 Ratio	2.1:1	2.2:1
# of T cells (x 10^9)	1.5	1.1
% Viability	99.0	99.0

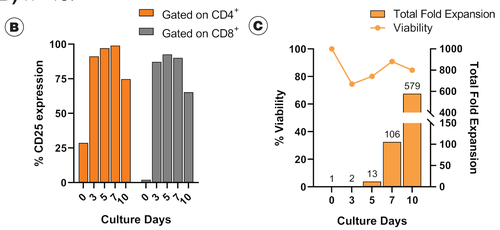

FIGURE 5. RoboSep™-C-Isolated T Cells Show Robust Activation and Expansion when Stimulated with ImmunoCult™ Human T Cell Activator in ImmunoCult™-XF T Cell Expansion Medium

T cells were isolated from fresh human peripheral blood Leukopak (2.7 x 10^9 start cells using RoboSep™-C). The isolated cells were stimulated with ImmunoCult™ Human CD3/CD28/CD2 T Cell Activator and cultured for 10 days in ImmunoCult™-XF T Cell Expansion Medium. Cells were subcultured at days 3, 5, and 7. **(A)** Start and isolated cell analysis. **(B)** Expression of activation marker CD25 assessed by flow cytometry and **(C)** total fold T cell expansion and viability assessed with NucleoCounter® NC-250™ over the 10-day expansion assay. Results from a single representative experiment are shown.

Summary

- RoboSep™-C enables automated large-scale T cell isolation in a closed system in just 75 minutes
- RoboSep™-C isolates T cells with high purity, recovery, and viability
- T cells isolated with RoboSep™-C are functional and show robust activation and expansion upon stimulation