Quick and Easy Isolation of Immune Cells From Large-Volume Samples

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ABSTRACT

Large-scale cell isolation is commonly performed in labs and core facilities for cell banking and drug discovery, and as a critical step in cell therapy manufacturing. Current methods can be a significant bottleneck in a lab's workflow, often requiring a full day for sample processing and cell isolation. To address this need, we have developed two methods: manual column-free isolation using the Easy 250 EasySep™ magnet, and automated cell isolation in a closed system using RoboSep™-C.

The Easy 250 magnet allows users to isolate cells from a full leukopak in under 30 minutes by simply pipetting out their target cells. Negative selection protocols have been optimized to achieve 95.3% T cell, 97.2% $CD4^+$ T cell, 91.9% $CD8^+$ T cell, 99.4% B cell, 96.5% NK cell, and 91.5% monocyte purities. Positive selection protocols obtain 95.4% $CD3^+$ cell, 94.4% $CD4^+$ T cell, 93.9% $CD8^+$ T cell, and 96.2% $CD14^+$ cell purities.

RoboSep™-C automates this cell isolation procedure, along with the cell washing steps for sample preparation, in as little as 50 minutes. The instrument features a scale tower, pump and clamp modules to direct fluid through defined paths, and a magnet for separation of labeled cells. The system uses a sterile single-use tubing set that incorporates a cartridge for cell washing and concentration, and a magnet chamber for cell separation. Starting with fresh leukopaks, we obtained 95.9% T cell, 95.8% $CD4^+$ T cell, 89.6% $CD8^+$ T cell, 92.6% monocyte, and 90.5% NK cell purities following negative selection.

These approaches offer efficient and user-friendly cell isolation that allows researchers to scale up their operations, and can be easily integrated upstream of existing workflows.

METHODS

1. Add EasySep™ Isolation Cocktail to single-cell suspension in a T-75 cm^2 flask.
   Incubate for 5 minutes.
2. Add EasySep™ RapidSpheres™ to cell suspension.
   Place the T-75 cm^2 flask in EasySep™ Magnet.
3. Incubate for 10 minutes.
   Pipette off desired fractions into a new T-75 cm^2 flask, place it in EasySep™ Magnet, and incubate for 5 minutes.
4. Pipette off desired fraction into a new tube.
   Cells are ready for downstream applications.

FIGURE 1. Typical Cell Isolation Protocol Using the Easy 250 EasySep™ Magnet.

To prepare starting samples for cell isolation, leukopaks are washed by diluting with an equal volume of EasySep™ Buffer and centrifuged at 300 x *g* for 10 minutes. Optionally, red blood cell (RBC) lysis can be performed using ammonium chloride. Washed cells are resuspended at 5 x 10^7 cells/mL and up to 225 mL for negative selection, or 1 x 10^8 cells/mL and up to 125 mL for positive selection. Unwanted cells (negative selection) or target cells (positive selection) are labeled using antibody complexes and magnetic particles and are separated in a T-75 cm^2 flask using the Easy 250 EasySep™ Magnet. Estimated time for the cell isolation protocol is 20 - 30 minutes.

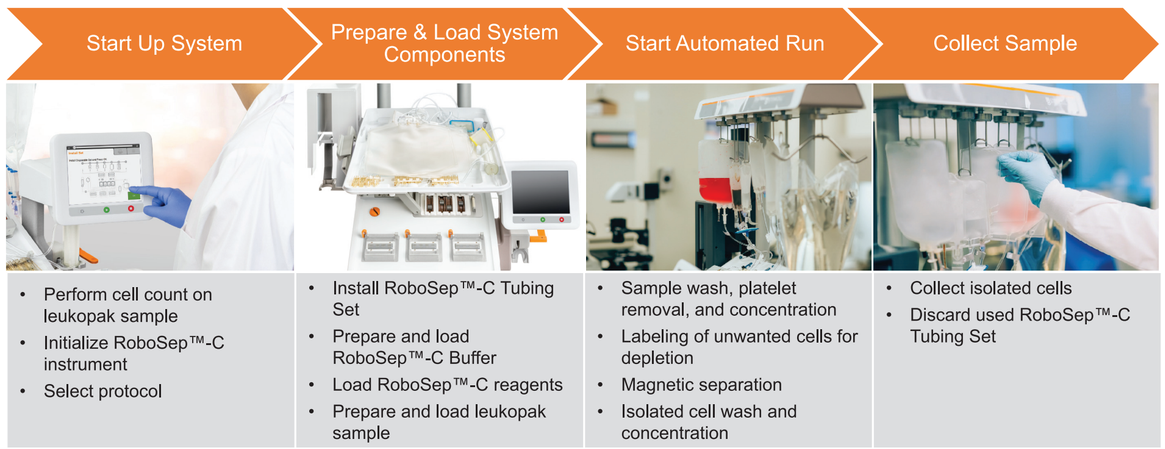

FIGURE 2. RoboSep™-C Protocol for Automated Cell Isolation.

Overview of instrument setup and automated protocol for isolation of target immune cells by negative selection. To initiate a run, all system components including the RoboSep™-C Tubing Set, Buffer, Cell Isolation reagents, and the starting leukopak (2.5 - 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 within the Cell Wash Cartridge of the tubing set. RoboSep™-C negative selection reagents label unwanted cells including RBCs and platelets for depletion. Estimated total protocol time is 75 minutes (25 minutes of hands-on time and 50 minutes for the automated run).

RESULTS

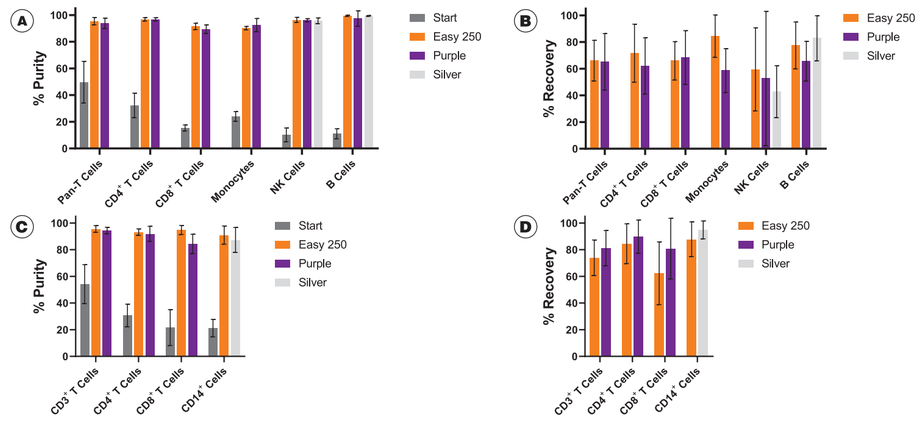

FIGURE 3. Isolation of High Purity Immune Cells from Large-Volume Samples Using the Easy 250 EasySep™ Magnet.

Immune cell subsets were isolated from processed leukopaks (washed only or washed and lysed) using various **(A - B)** negative selection or **(C - D)** positive selection EasySep™ kits. Isolations were performed using the Easy 250 EasySep™ magnet and compared to established small volume protocols using the EasySep™ magnet (purple) or "The Big Easy" EasySep™ Magnet (silver). **(A, C)** Purities of T cells ($CD3^+$), $CD4^+$ T cells ($CD4^+CD3^+$), $CD8^+$ T cells ($CD8^+CD3^+$), monocytes ($CD14^+$), NK cells ($CD56^+CD3^-$), or B cells ($CD20^+CD19^+$) within the viable $CD45^+$ population were assessed by flow cytometry. **(B, D)** 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%. Data are shown as mean ± SD; n = 4 – 12 for each cell isolation kit.

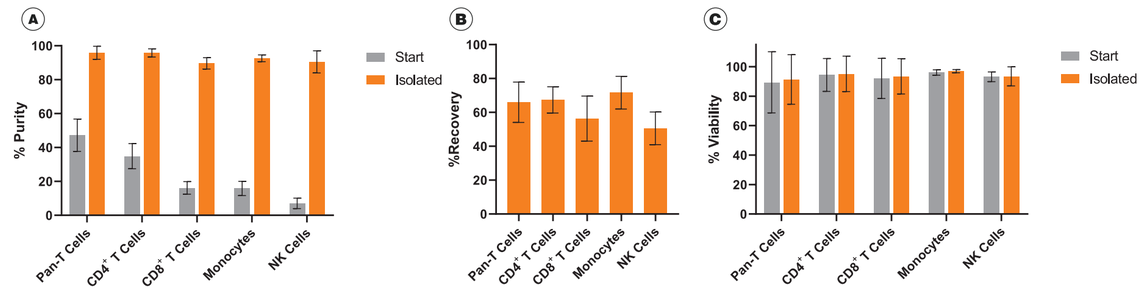

FIGURE 4. RoboSep™-C Enables Large-Scale Automated Negative Selection of Immune Cells with High Purity, Recovery, and Viability.

Pan-T cells, $CD4^+$ T cells, $CD8^+$ T cells, monocytes, or NK cells were isolated from fresh human peripheral blood leukopaks (2.5 - 20 x 10^9 start cells) using the RoboSep™-C system. **(A)** Purities of T cells ($CD3^+$), $CD4^+$ T cells ($CD4^+CD3^+$), $CD8^+$ T cells ($CD8^+CD3^+$), monocytes ($CD14^+CD16^-$), or NK cells ($CD56^+CD3^-$) within the viable $CD45^+$ population were 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)** Start leukopak and post-isolation viability were assessed by staining with the cell viability dye DRAQ7™ and analyzed by flow cytometry. Data are shown as mean ± SD; n = 8 – 20 for each cell isolation kit.

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

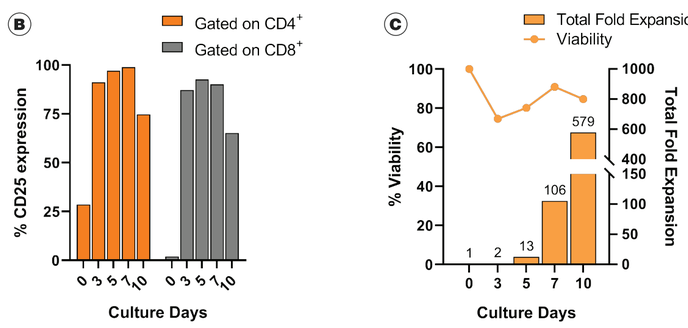

FIGURE 5. RoboSep™-C-Isolated T Cells Show Robust Activation and Expansion when Stimulated with ImmunoCult™ Human T Cell Activator in ImmunoCult™-XF T Cell Expansion Medium.

T cells were isolated from fresh human peripheral blood leukopak (2.7 x 10^9 start cells). 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)** Analysis of start and isolated cell samples. **(B)** Expression of the activation marker CD25 was assessed by flow cytometry and **(C)** total T cell fold expansion and viability were assessed using a NucleoCounter® NC-250™ over the 10-day expansion assay. Results from a single representative experiment are shown.

Summary

- The Easy 250 EasySep™ Magnet and RoboSep™-C enable large-volume isolation of immune cells with high purity and recovery
- Isolations using the Easy 250 EasySep ™ Magnet can be completed in less than 30 minutes, while the RoboSep™-C automates this procedure along with the upstream and downstream cell washing steps in a 50-minute protocol
- T cells isolated with RoboSep™-C are functional and show robust activation and expansion upon stimulation