Fully immunomagnetic isolation of untouched purified lymphocyte populations directly from whole blood without the need for red blood cell sedimentation, hypotonic lysis or density gradient centrifugation in just 25 minutes

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Introduction

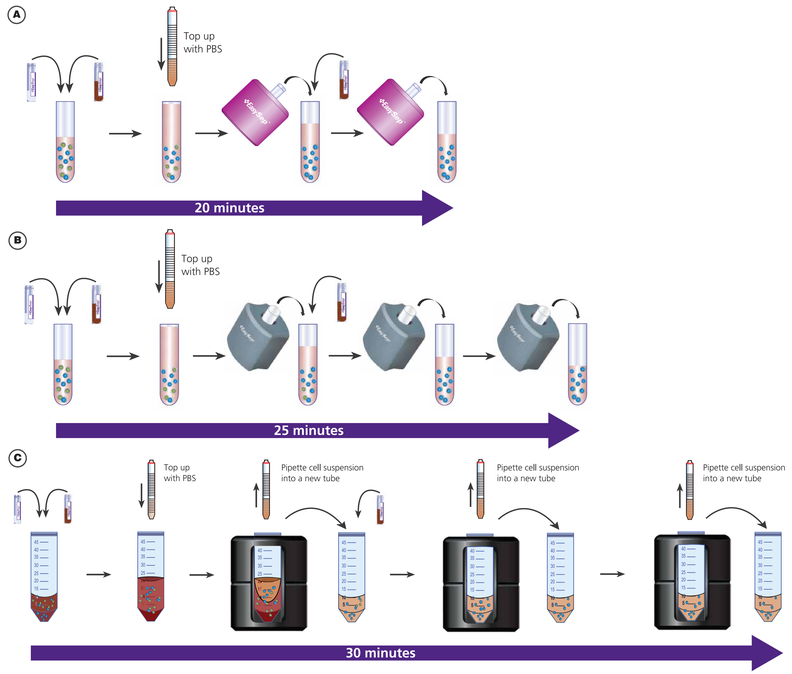
Human whole blood is made up of 93 - 96% red blood cells (RBCs), 4 - 7% platelets and 0.1 - 0.2% leukocytes consisting mainly of granulocytes, lymphocytes and monocytes. Due to the low frequency of lymphocyte subsets within whole blood, their isolation typically requires a pre-processing step such as Lymphoprep™ or hypotonic lysis to deplete RBCs prior to cell isolation. We have therefore developed a new, fully immunomagnetic method for the negative selection of untouched cells directly from whole blood, without the need for any pre-processing steps. The EasySep™ Direct procedure involves labeling RBCs, platelets and unwanted leukocytes present in human whole blood with antibodies and magnetic particles. The magnetically-labeled unwanted cells are separated from the untouched desired cells using an EasySep™ magnet by simply pouring or pipetting the desired cells into a new tube. In just 25 minutes, purities of 92 - 99% total lymphocytes, 93 - 98% T cells, 87 - 97% CD4$^+$ T cells, 75 - 91% CD8$^+$ T cells, 91 - 99% B cells, 87 - 98% naïve B cells and 65 - 84% NK cells can be achieved with little to no residual RBC contamination. The isolation of cells directly from whole blood will enable rapid access to highly purified cells immediately ready for downstream functional assays with minimal sample handling.

Methods

FIGURE 1: EasySep™ Direct compatible EasySep™ magnets

The EasySep™ Direct procedure is compatible with the A) EasySep™ Magnet (Catalog #18000), B) "The Big Easy" EasySep™ Magnet (Catalog #18001), C) EasyEights™ EasySep™ Magnet (Catalog #18103) and the D) Easy 50 EasySep™ Magnet (Catalog #18002).

FIGURE 2: EasySep™ Direct protocols for human whole blood negative selection negative selection without RBC lysis

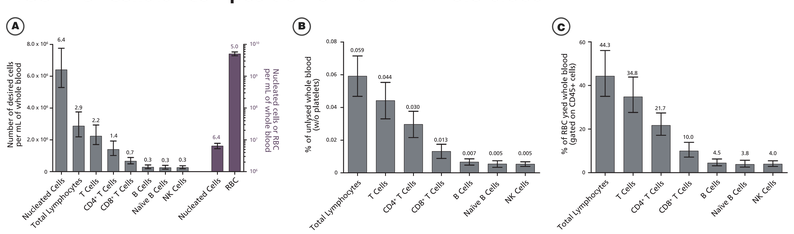


EasySep™ Direct protocol for the isolation of untouched lymphocyte subsets directly from whole blood in as little as 20 minutes. Starting with human whole blood, the EasySep™ Direct procedure involves the addition of the EasySep™ Direct Cocktail and EasySep™ Direct RapidSpheres™ and either A) two or B and C) three magnetic separations depending on the EasySep™ magnet used. The sample is either poured or pipetted off from the magnet.

TABLE 1: EasySep™ Direct lymphocyte isolation protocol comparison

EasySep™ Magnet	Sample Volume Range (mL)	Top up sample with PBS without Ca^{2+} and Mg^{2+}	Total Protocol Time
Purple	0.5 - 1.5	2.5 mL	~20 minutes
Silver	1.0 - 7.0	Double volume for samples ≤ 5 mL Top up to 10 mL for samples > 5 mL	~25 minutes
EasyEights™ (4 mL)	0.5 - 1.5	2.5 mL	~25 minutes
EasyEights™ (14 mL)	1.0 - 7.0	Double volume for samples ≤ 5 mL Top up to 10 mL for samples > 5 mL	~25 minutes
Easy 50	7.0 - 30	Double volume for samples ≤ 25 mL Top up to 50 mL for samples > 25 mL	~30 minutes

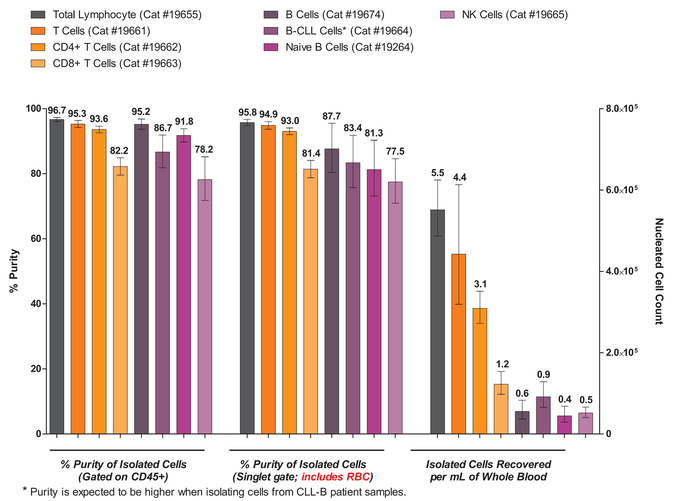
FIGURE 3: Cellular composition of human whole blood



Number and relative frequency of lymphocytes in human whole blood. A) Absolute numbers of peripheral blood nucleated cells, red blood cells and lymphocyte subsets in human whole blood. B) Frequency of lymphocyte subsets in unlysed human whole blood. C) Frequency of lymphocyte subsets in RBC lysed whole blood gated on CD45$^+$ cells.

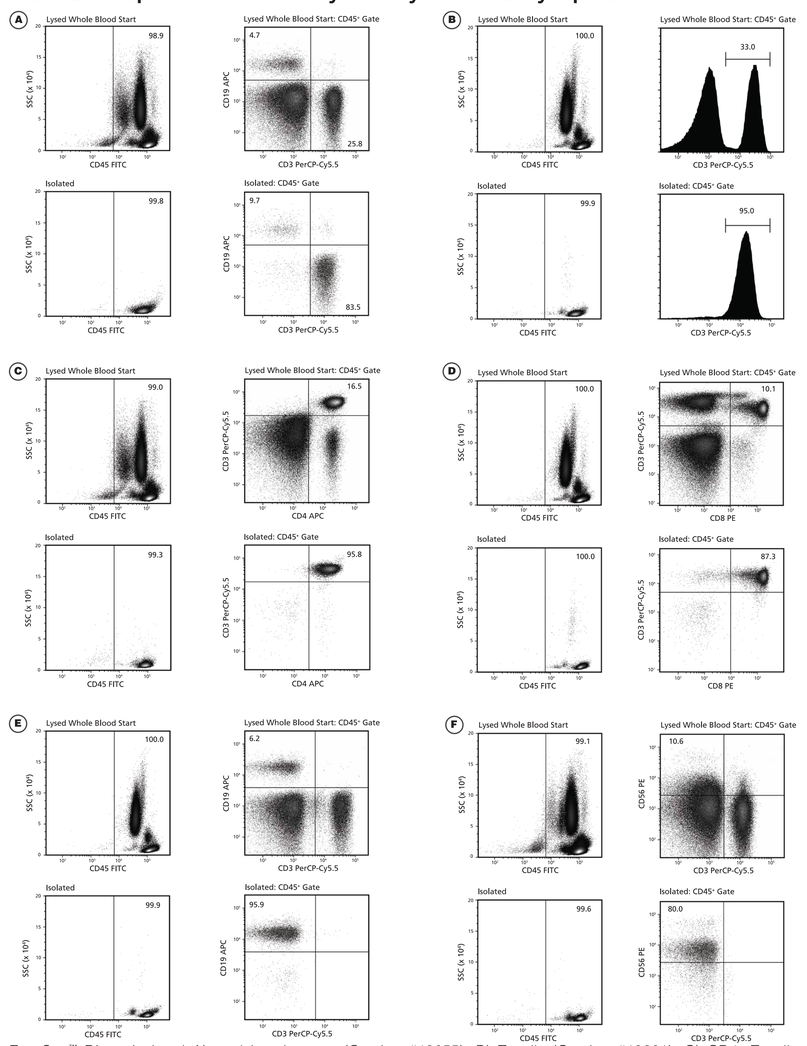
Results

FIGURE 4: EasySep™ Direct performance summary



EasySep™ Direct purity and total cell recovery for lymphocyte isolation kits. Purity of isolated cells gated on CD45$^+$ nucleated cells and purity of isolated cells including residual debris, platelets and RBCs is shown. Average purity is indicated above each bar with a 95% confidence interval. Total cell recovery is indicated as the number of cells recovered per mL of starting whole blood volume.

FIGURE 5: Representative flow cytometry data of EasySep™ Direct isolated cells



EasySep™ Direct isolated A) total lymphocytes (Catalog #19655), B) T cells (Catalog #19661), C) CD4$^+$ T cells (Catalog #19662), D) CD8$^+$ T cells (Catalog #19663), E) B cells (Catalog #19674) and F) NK cells (Catalog #19665) were stained using fluorochrome conjugated antibodies and purity assessed by flow cytometry. Red blood cells in the human whole blood start samples were lysed using ammonium chloride to determine starting cell frequencies.

Conclusions

- New fully immunomagnetic method for the negative selection of human lymphocyte subsets directly from whole blood with little to no residual RBC contamination.
- >99.9% RBC depletion without the need for density gradient centrifugation, sedimentation or lysis.
- Purities of greater than 95% can be achieved.
- Fast, easy-to-use and column-free isolation of untouched lymphocyte subsets in as little as 20 minutes.