Easy 250 EasySep™ Magnet: A Novel Magnetic Platform for Large-Volume Cell Isolation from Whole Blood and Leukapheresis Packs

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INTRODUCTION

Isolating cells from large-volume samples such as leukapheresis or whole blood bags is a common procedure that precedes many immunological studies. Working with large volumes can be challenging, as the sample often needs to be split and processed in parallel, which can be tedious and may delay downstream studies. The Easy 250 EasySep™ Magnet simplifies cell separation when processing up to 225 mL of leukapheresis sample (Leukopak) or 125 mL of whole blood, and is based on column-free immunomagnetic EasySep™ cell isolation technology. Negative and positive cell isolation protocols were optimized for various cell types and all protocols can be completed in 20 - 26 minutes.

METHODS

EasySep™ Negative Selection for T Cells, CD4⁺ T Cells, CD8⁺ T Cells, and Monocytes from Leukopaks

Leukopaks were diluted with an equal volume of EasySep™ Buffer and centrifuged at 300 x *g* for 10 minutes, with low brake. After the supernatant was removed, ~20 mL of ammonium chloride was added to the cell pellet and the cell suspension was incubated on ice for 10 - 15 minutes. The sample was then topped up with EasySep™ Buffer and centrifuged at 300 x *g* for 10 minutes, low brake. After removing the supernatant, the sample was resuspended in EasySep™ Buffer and centrifuged for 10 minutes at 120 x *g*, with no brake. The cells were resuspended to the cell concentration recommended in the Product Information Sheet (PIS) prior to immunomagnetic cell isolation. Unwanted cells were targeted for depletion using antibody complexes and magnetic particles and separated in either a T-75 cm^2 flask using the Easy 250 EasySep™ Magnet (Figure 1), or in a 5 mL tube using the purple EasySep™ Magnet for comparison.

EasySep™ Positive Selection for CD3⁺, CD8⁺ and CD14⁺ Cells from Leukopaks

Leukopaks were diluted with an equal volume of EasySep™ Buffer and centrifuged at 300 x *g* for 10 minutes, low brake. After the supernatant was removed, cells were resuspended in EasySep™ Buffer and a slow spin was performed at 120 x *g* for 10 minutes, no brake. The cells were resuspended to the cell concentration recommended in the PIS prior to immunomagnetic cell isolation. The cells of interest were labeled with antibody complexes and magnetic particles. Labeled cells were separated from unlabeled cells in a T-75 cm^2 flask using the Easy 250 EasySep™ Magnet (Figure 1), or in a 5 mL tube using the purple EasySep™ Magnet, or a 14 mL tube using "The Big Easy" EasySep™ Magnet, for comparison.

EasySep™ Red Blood Cell (RBC) Depletion and Isolation of Peripheral Blood Mononuclear Cells (PBMCs) from Whole Blood

EDTA was added to whole blood to a final concentration of 6 mM prior to cell isolation. Unwanted cells were targeted for depletion directly from whole blood using antibody complexes and magnetic particles and separated in a T-75 cm^2 flask using the Easy 250 EasySep™ Magnet (Figure 1), or in a 5 mL tube using the purple EasySep™ Magnet for comparison.

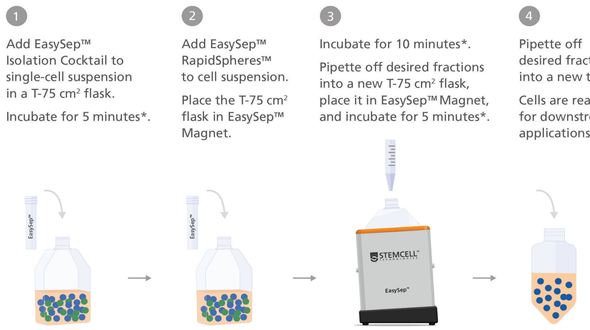

FIGURE 1. Immunomagnetic Cell Isolation Using the Easy 250 EasySep™ Magnet

RESULTS

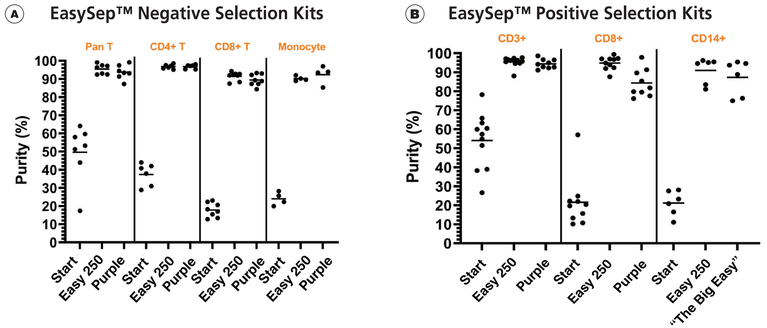

FIGURE 2. Isolation of Cells from Large-Volume Samples Using Easy 250 EasySep™ Magnet Results in Highly Pure T Cell and Monocyte Populations

Immune cell subsets were isolated from Leukopaks (negative selection: 40 - 225 mL; positive selection: 40 - 125 mL) using various EasySep™ kits. **(A)** Average purities (± SD) obtained for each negative cell isolation kit are as follows: 96.5 ± 2.6% (n = 12, Easy 250 magnet) and 95.6 ± 4.0% (n = 12, purple magnet) using EasySep™ Human T Cell Isolation Kit; 96.5 ± 1.7% (n = 11, Easy 250 magnet) and 96.5 ± 1.6% (n = 11, purple magnet) using EasySep™ Human CD4+ T Cell Isolation Kit; 88.5 ± 5.5% (n = 15, Easy 250 magnet) and 89.6 ± 3.9% (n = 13, purple magnet) using EasySep™ Human CD8+ T Cell Isolation Kit; 86.6 ± 5.0% (n = 14, Easy 250 magnet) and 88.3 ± 4.0% (n = 13, purple magnet) using EasySep™ Human Monocyte Isolation Kit. **(B)** Average purities (± SD) obtained for each positive selection kit are as follows: 95.9 ± 2.8% (n = 14, Easy 250 magnet) and 95.0 ± 2.4% (n = 10, purple magnet) using EasySep™ Human CD3 Positive Selection Kit II; 95.9 ± 3.0% (n = 14, Easy 250 magnet) and 88.8 ± 4.8% (n = 10, purple magnet) using EasySep™ Human CD8 Positive Selection Kit II; 93.9 ± 4.9% (n = 12, Easy 250 magnet) and 94.3 ± 4.4% (n = 7, "The Big Easy" magnet) using EasySep™ Human CD14 Positive Selection Kit II.

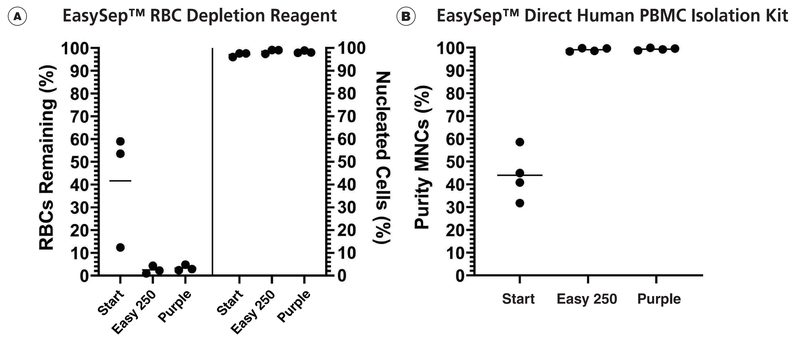

FIGURE 3. The Easy 250 EasySep™ Magnet Can Be Used to Deplete RBCs or Isolate PBMCs from Large-Volume Whole Blood Samples

Large-volume (25 - 125 mL) whole blood samples were processed using the Easy 250 EasySep™ Magnet and various EasySep™ kits. For comparison, 1 mL whole blood was processed using the purple EasySep™ magnet. **(A)** EasySep™ RBC Depletion Reagent was used to obtain a highly purified nucleated cell fraction free of RBCs. The percentage of RBCs remaining following depletion (± SD) were 2.5 ± 1.7% (n = 3, Easy 250 magnet) and 3.35 ± 1.3% (n = 3, purple magnet). **(B)** EasySep™ Direct Human PBMC Isolation Kit was used to obtain a highly purified PBMC fraction. Average mononuclear cell (MNC) purities (± SD) of 99.0 ± 0.5% (n = 5, Easy 250 magnet) and 99.4 ± 0.4% (n = 5, purple magnet) were obtained.

TABLE 1. Number of Cells Recovered from Large-Volume Samples Following Isolation Using the Easy 250 EasySep™ Magnet

		# Cells recovered per 5 x 10^7 processed LP start cells				# Cells recovered per 1 x 10^8 processed LP start cells			# Nucleated cells recovered/mL of WB	# PBMCs recovered/mL of WB
	Cell Type: Catalog #:	T Cell 17951	CD4+ T 17952	CD8+ T 17953	Monocyte 19359	CD3+ 17851	CD8+ 17853	CD14+ 17858	RBC Depletion 18170	PBMC 19654
Easy 250 Magnet	Average	1.74 x 10^7	1.19 x 10^7	5.26 x 10^6	6.24 x 10^6	3.89 x 10^7	1.28 x 10^7	1.87 x 10^7	3.90 x 10^6	1.36 x 10^6
	SD	6.33 x 10^6	4.29 x 10^6	1.88 x 10^6	3.17 x 10^6	9.51 x 10^6	5.84 x 10^6	5.39 x 10^6	7.82 x 10^5	2.39 x 10^5
Purple Magnet	Average	1.55 x 10^7	1.16 x 10^7	3.97 x 10^6	5.00 x 10^6	4.35 x 10^7	2.28 x 10^7	1.89 x 10^7	3.91 x 10^6	1.26 x 10^6
	SD	8.88 x 10^6	4.53 x 10^6	1.62 x 10^6	2.05 x 10^6	1.47 x 10^7	3.35 x 10^7	1.07 x 10^7	6.41 x 10^5	3.34 x 10^5

SD = standard deviation; LP = leukapheresis pack; WB = whole blood

Summary

- The new Easy 250 EasySep™ Magnet is compatible with EasySep™ isolation kits
- Cell purity and recovery using the Easy 250 EasySep™ Magnet are comparable to the purple EasySep™ Magnet
- Whole blood or Leukopak volumes up to 125 mL and 225 mL, respectively, can be processed in 20 - 26 minutes