

A simple and rapid method for the isolation of untouched human memory CD8⁺ T cells

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Abstract

Memory CD8⁺ T cells are long-lived antigen-specific cells that persist after clearance of infection. Functionally, they are distinguished from naïve CD8⁺ T cells by their less extensive signal requirement for activation and ability to respond quickly to recall antigens and secrete a broad repertoire of cytokines. In addition, the two populations are phenotypically distinct, with naïve cells expressing CD45RA, and memory cells expressing CD45RO, a marker indicative of previous activation. Current protocols for the isolation of memory CD8⁺ T cells are time-consuming and require the use of columns. We have developed a new kit for easy and rapid isolation of memory CD8⁺ T cells from PBMCs by immunomagnetic, column-free, cell separation (EasySep™). Non-CD8⁺ T cells and CD45RA positive CD8⁺ T cell subsets are targeted for depletion by bispecific tetrameric antibody complexes crosslinked to dextran-coated magnetic particles. The labeled cells are separated using an EasySep™ magnet and the desired fraction is poured off. The procedure is performed in 30 minutes and can be fully automated using RoboSep™. The mean enrichment purity and recovery of CD45RO⁺CD45RA⁻CD8⁺ T cells is 86% ± 4, and 23% ± 11, respectively. The kit provides a simple and efficient means of isolating untouched human memory CD8⁺ T cells that are ideal for studies in signal transduction, activation, cytokine expression, and response to infectious disease.

Methods

Preparation of Starting Cell Suspension

A mononuclear cell suspension was prepared from whole blood or buffy coat by Ficoll-Paque PLUS™ density separation. Alternatively, peripheral blood apheresis (Leukopak) cells were used following red blood cell lysis and one or more washes to remove platelets. Start cells were resuspended at 5x10⁷ cells/mL in PBS + 2% FBS and 1mM EDTA.

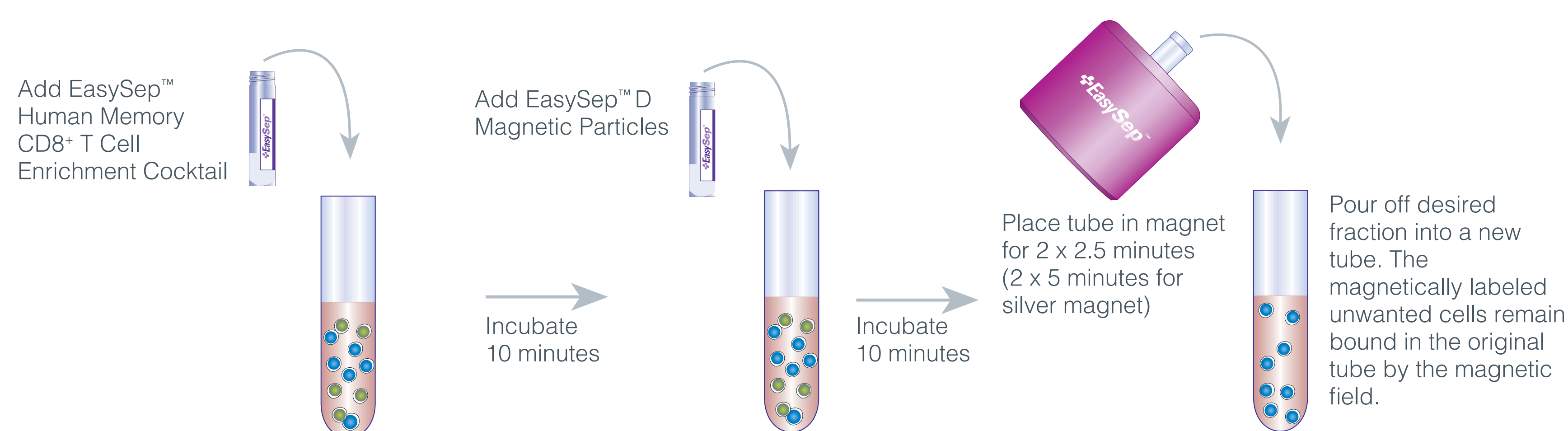
TABLE 1: Antibodies used to deplete non-CD8 T cells and unwanted CD8⁺ T cell subsets in the EasySep™ Memory CD8⁺ T cell enrichment kit

Cell population targeted for depletion	Antibodies used in cocktail to target unwanted cells
Non-CD8 ⁺ T cells	CD4, CD14, CD16, CD19, CD20, CD34, CD36, CD56, CD61, CD66b, CD123, TCR $\gamma\sigma$, GlyA
Non-memory CD8 ⁺ T cells	CD45RA

Purity Assessment

EasySep™ isolated memory CD8⁺ T cells were assessed by flow cytometry using a combination of antibodies specific for naïve, effector and memory cells. Memory CD8⁺ T cells are CD8⁺CD45RO⁺CD45RA⁻.

FIGURE 1: EasySep™ procedure for column-free enrichment of memory CD8⁺ T cells from human PBMCs



This procedure can be fully automated using RoboSep™.

Results

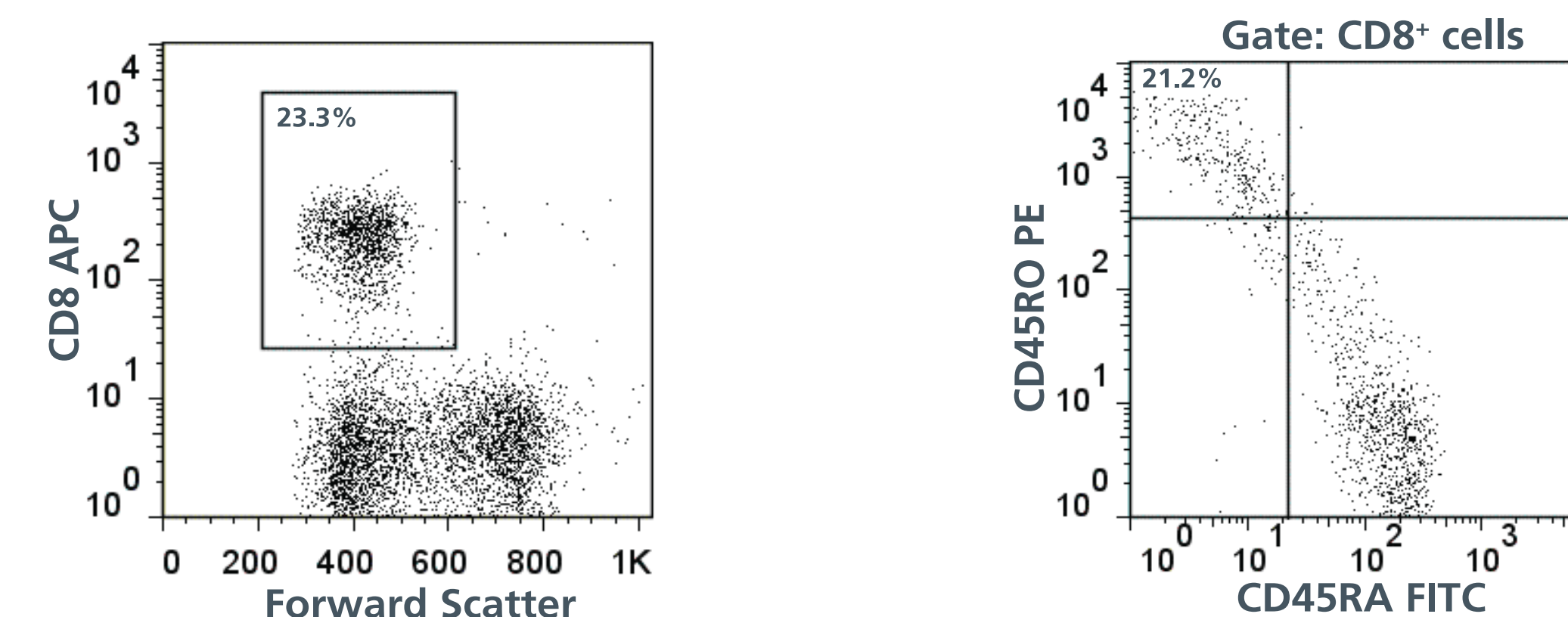
TABLE 2: Purity and recovery of memory CD8⁺ T cells enriched from PBMC by EasySep™ or RoboSep™

Method	n	% Purity	% Recovery
EasySep™	52	85.9 ± 4.4	22.7 ± 11.2
RoboSep™	10	85.0 ± 4.5	22.0 ± 8.4

Purities determined by flow cytometry. Memory CD8⁺ T cells are defined as CD8⁺CD45RO⁺CD45RA⁻. Values are expressed as means ± 1 SD.

FIGURE 2: Flow cytometric assessment of memory CD8⁺ T cells before and after enrichment using EasySep™

Start: 4.9% CD8⁺CD45RO⁺CD45RA⁻ cells



Enriched: 88.8% CD8⁺CD45RO⁺CD45RA⁻ cells

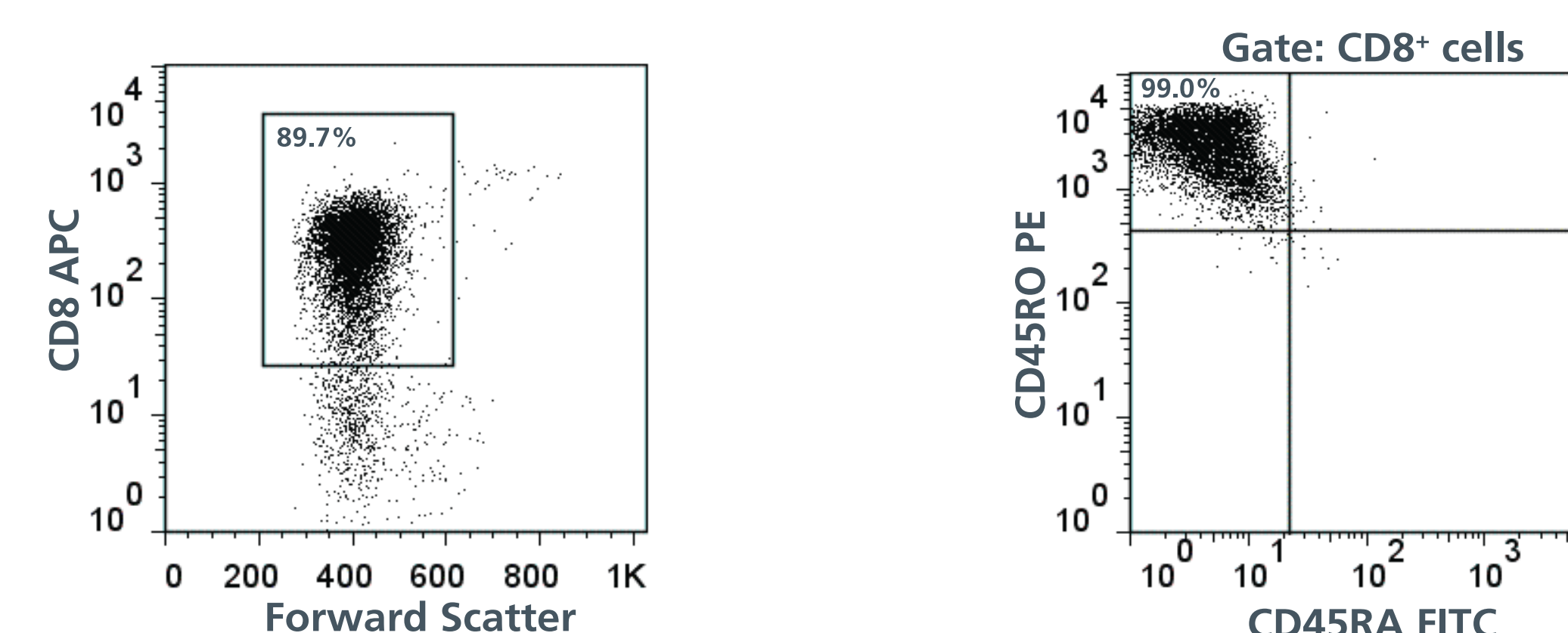


TABLE 3: Comparison of memory CD8⁺ T cell isolation protocols using EasySep™/RoboSep™ or the column-based competitor kits

	EasySep™	RoboSep™	Competitor
Total time	25-30 min	44 min	~1 hr 15 min
Columns	0	0	1
Centrifugations	0	0	3
Isolation method	untouched	untouched	untouched

Conclusions

- Isolate untouched human memory CD8⁺ T cells from PBMC in 30 minutes.
- Memory CD8⁺ T cell isolation can be fully automated with RoboSep™.
- Average purities and recoveries for memory CD8⁺ enrichments are 85.9% ± 4.4 and 22.7% ± 11.2, respectively.
- EasySep™ isolated memory CD8⁺ T cells are untouched and are ideal for functional T cell studies.