

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

M. Fairhurst, V. Posarac, K. McQueen, and T. Thomas
STEMCELL Technologies Inc., Vancouver, BC, Canada

Introduction

CD8⁺ T cells play a central role in the adaptive immune response to intracellular pathogens. The human CD8⁺ T cell pool is comprised of several phenotypically and functionally distinct sub-populations, including naïve cells that have not encountered antigen and express CD45RA and CCR7, and previously activated memory cells, primarily defined by CD45RO expression. Current protocols for the isolation of these populations are time-consuming and require the use of columns. We have developed two new kits for easy and rapid isolation of naïve (CD45RA⁺CCR7⁺) and memory (CD45RO⁺) CD8⁺ T cells from peripheral blood mononuclear cells (PBMC) by immunomagnetic, column-free cell separation (EasySep™). Non-CD8⁺ T cells and unwanted CD8⁺ T cell subsets are targeted for depletion by antibody complexes crosslinked to dextran-coated magnetic particles. The labeled cells are separated using an EasySep™ magnet and the desired fraction is poured off. Each procedure is performed in less than an hour and can be fully automated using RoboSep™. The mean enrichment purities for the naïve and memory CD8⁺ T cell kits are 89 ± 3% and 86 ± 4%, respectively. The kits provide a simple and efficient means of isolating untouched human naïve and 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, for memory CD8⁺ T cell isolations, 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.

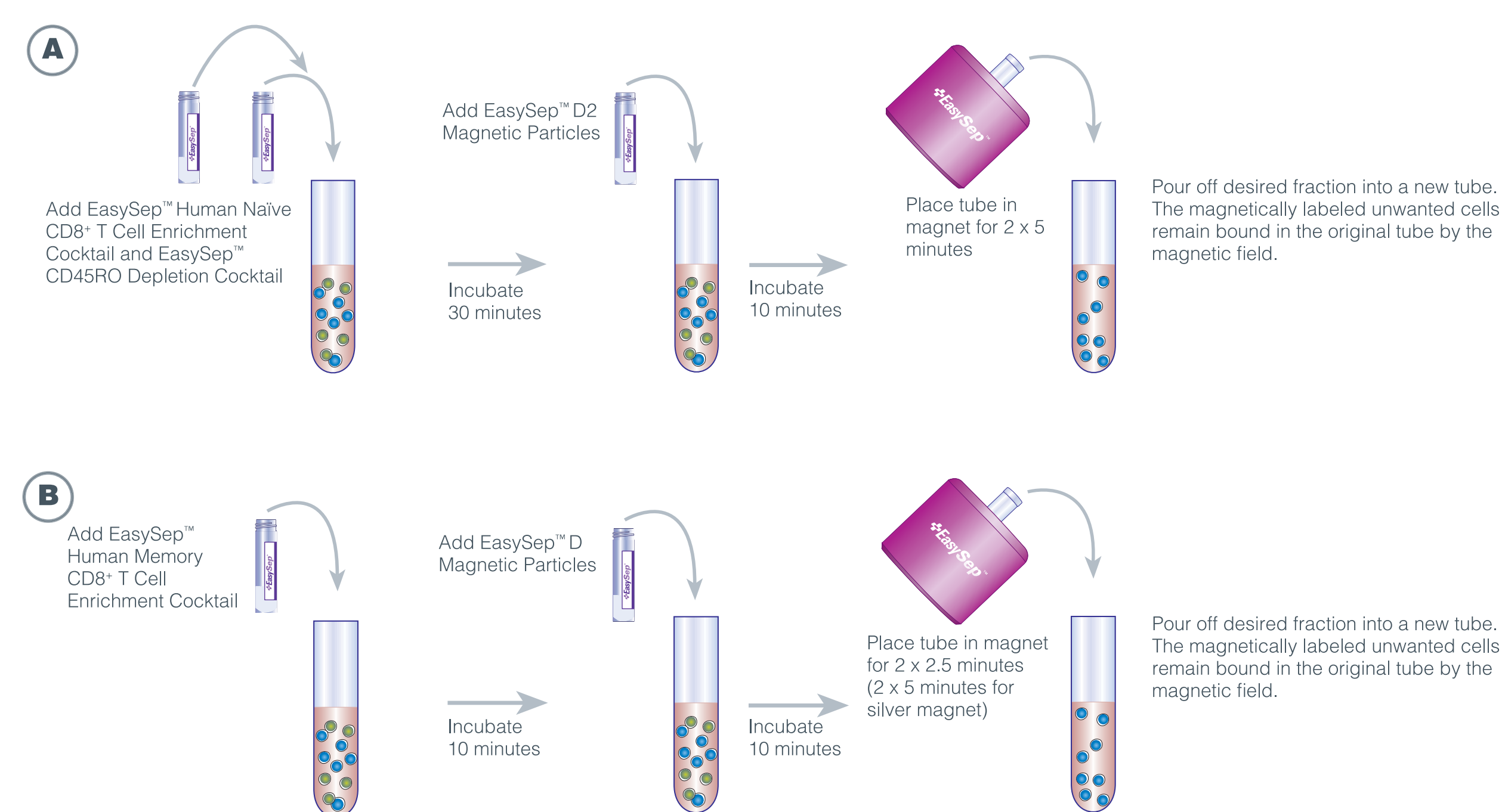
TABLE 1: Antibodies used to deplete non-CD8⁺ T cells and unwanted CD8⁺ T cell subsets in the EasySep™ Naïve CD8⁺ T Cell and Memory CD8⁺ T Cell enrichment kits

EasySep™ kit	Cell population targeted for depletion	Antibodies used in cocktail to target unwanted cells
Naïve CD8 ⁺ T Cell	Non-CD8 ⁺ T cells	CD4, CD14, CD16, CD19, CD20, CD36, CD123, TCRγδ, GlyA
	Non-naïve CD8 ⁺ T cells	CD45RO, CD56, CD57, CD94, CD244
Memory CD8 ⁺ T Cell	Non-CD8 ⁺ T cells	CD4, CD14, CD16, CD19, CD20, CD34, CD36, CD56, CD61, CD66b, CD123, TCRγδ, GlyA
	Non-memory CD8 ⁺ T cells	CD45RA

Purity Assessment

EasySep™ isolated naïve and memory CD8⁺ T cells were assessed by flow cytometry using a combination of antibodies specific for naïve, effector, and memory cells. Naïve CD8⁺ T cells are defined as CD8⁺CD45RA⁺CCR7⁺ and CD45RO⁻CD56⁻CD57⁻. Memory CD8⁺ T cells are CD8⁺CD45RO⁺CD45RA⁻.

FIGURE 1: EasySep™ procedure for column-free enrichment of naïve CD8⁺ T cells (A) and memory CD8⁺ T cells (B) from human PBMC



These procedures can be fully automated using RoboSep™.

Results

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

	Naïve CD8 ⁺ T Cell Kit			Memory CD8 ⁺ T Cell Kit		
	n	% Purity	% Recovery	n	% Purity	% Recovery
EasySep™	8	89.3 ± 2.6	64.2 ± 25.1	52	85.9 ± 4.4	22.7 ± 11.2
RoboSep™	4	89.0 ± 5.5	62.7 ± 12.5	10	85.0 ± 4.5	22.0 ± 8.4

Values are expressed as means ± 1 SD.

FIGURE 2: Flow cytometric assessment of naïve CD8⁺ T cells (A) and memory CD8⁺ T cells (B) before and after enrichment using EasySep™

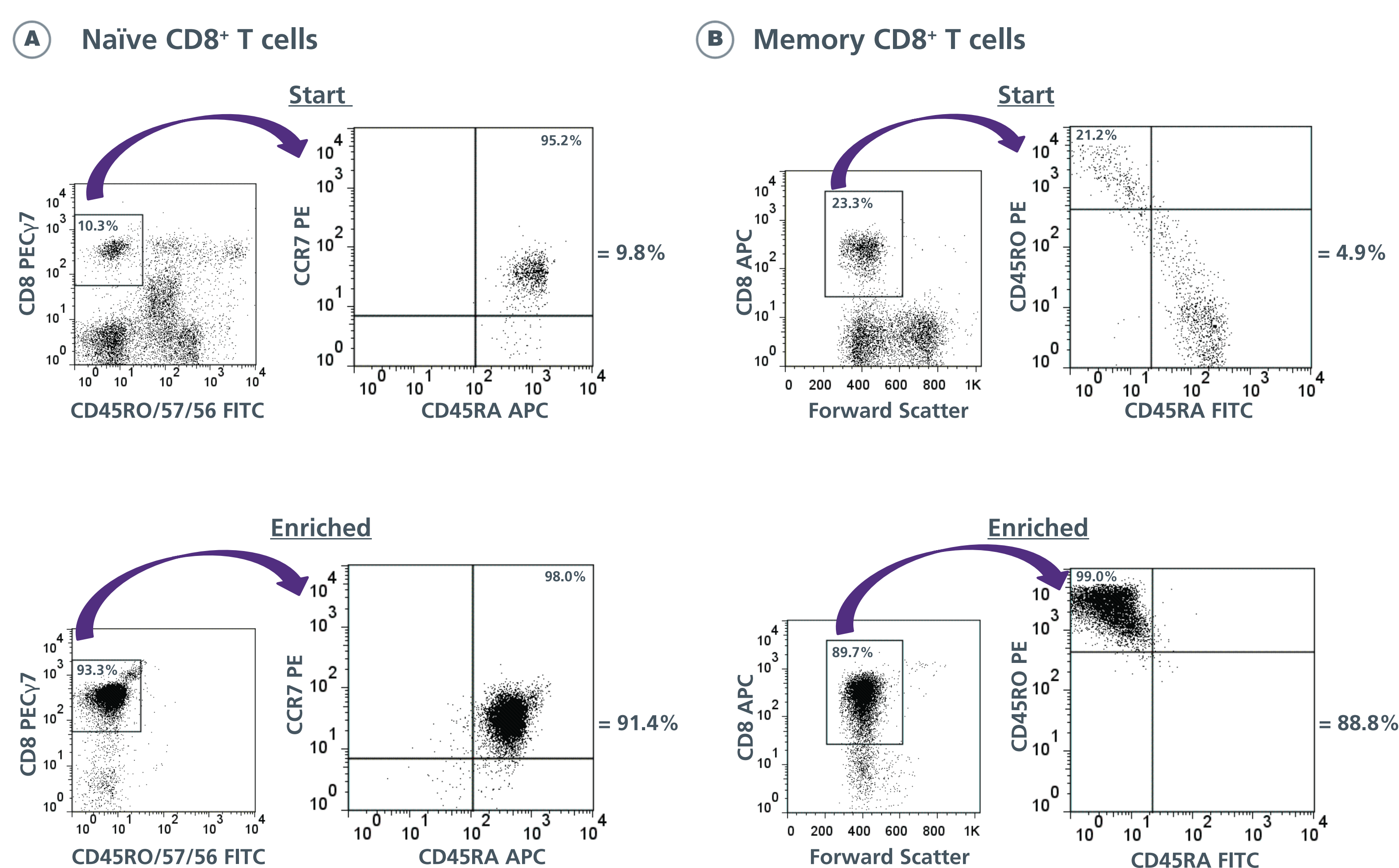


TABLE 3: Comparison of protocols for isolating naïve and memory CD8⁺ T cells using EasySep™/RoboSep™ or column-based competitor kits

	Naïve CD8 ⁺ T Cell Kit			Memory CD8 ⁺ T Cell Kit		
	STEMCELL		Competitor	STEMCELL		Competitor
	EasySep™	RoboSep™	Column	EasySep™	RoboSep™	Column
Total Time	~50 min	62 min	~1 hr 45 min	~25 - 30 min	44 min	~1 hr 15 min
Isolation Method	untouched	untouched	positive selection	untouched	untouched	untouched

Conclusions

- Isolate untouched naïve CD8⁺ T cells and memory CD8⁺ T cells from PBMC in under 60 minutes each.
- Naïve and memory CD8⁺ T cell isolation can be fully automated with RoboSep™.
- Average purities for naïve CD8⁺ T cell enrichments are 89.3 ± 2.6.
- Average purities for memory CD8⁺ T cell enrichments are 85.9 ± 4.4.
- EasySep™ isolated naïve and memory CD8⁺ T cells are untouched and are ideal for functional T cell studies.