

A Rapid Method for the Isolation of Untouched Naïve CD8⁺ T Cells from Peripheral Blood Mononuclear Cells

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Summary

Naïve and memory CD8⁺ T cells can most simply be distinguished by the reciprocal expression of CD45RA and CD45RO. A naïve CD8⁺ T cell is a mature cell that has been released by the thymus but has not yet encountered its cognate antigen. Naïve CD8⁺ T cells (CD45RA⁺/CD45RO⁻/CCR7⁺/CD62L⁺) migrate through the secondary lymphoid organs seeking antigens presented by MHC class I expressing cells. Once they encounter antigens they become activated through the T cell receptor and proliferate to generate CD45RO⁺ effector CD8⁺ T cells.

We describe an easy and rapid method for the isolation of untouched naïve CD8⁺ T cells from peripheral blood mononuclear cells (PBMC). Briefly, PBMCs are isolated by Ficoll-Paque PLUS density gradient sedimentation. Naïve CD8⁺ T cells are then enriched using immunomagnetic, column-free, negative selection (EasySep™). Unwanted cells are removed using antibody complexes recognizing non-naïve CD8⁺ T cells (Table 1) and dextran-coated magnetic particles. Following a 30 minute antibody cocktail incubation, magnetic particles are added and incubated for 10 minutes. Labeled cells are separated by placing the tube in an EasySep™ magnet for two 5 minute separations. Desired cells are then poured off into a new tube (Fig. 1). This procedure can be fully automated using RoboSep™. This simple method to isolate naïve CD8⁺ T cells will be invaluable to researchers studying T cell differentiation, autoimmunity and infectious diseases.

Methods

Sample Source

A mononuclear cell suspension was prepared from fresh whole blood or buffy coat by Ficoll-Paque PLUS™ density separation.

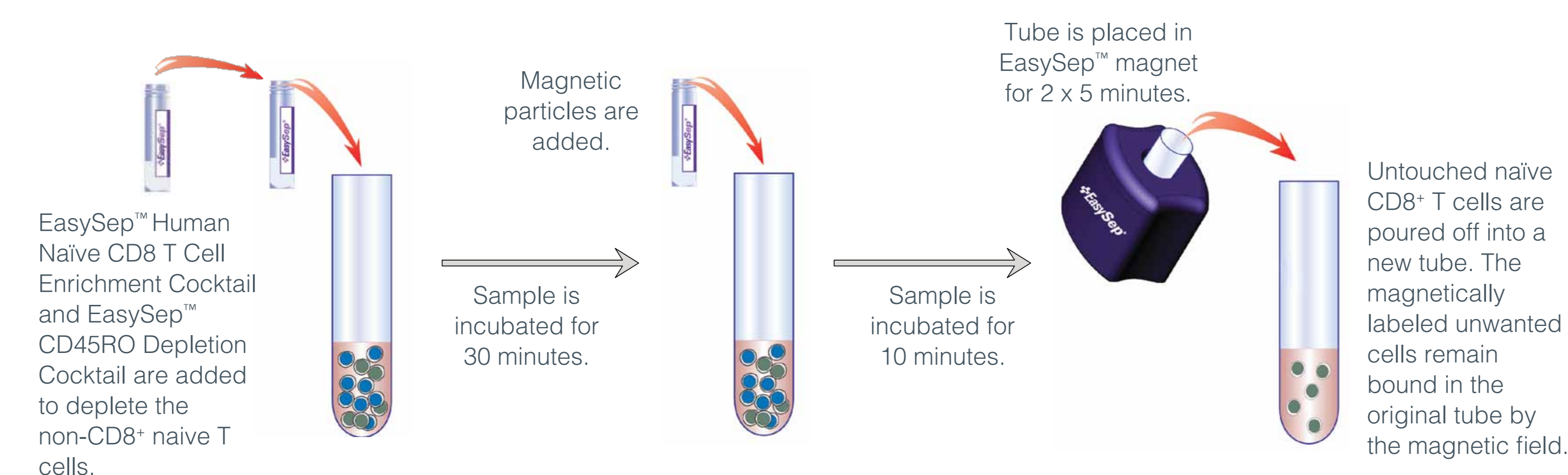
TABLE 1: Antibodies used to deplete unwanted cells in the EasySep™ Naïve CD8⁺ T Cell Enrichment Kit

Cell population targeted for depletion	Antibodies used in cocktail to target unwanted cells
Non-CD8 T cells	CD4, CD14, CD16, CD19, CD20, CD36, CD123, TCRγδ, GlyA
Non-naïve CD8 T cells	CD45RO, CD56, CD57, CD94, CD244

Purity Assessment

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

FIGURE 1: Manual EasySep™ protocol diagram



This procedure can be fully automated using RoboSep™.

Results

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

Method	n	% Naïve CD8 ⁺ T start	% Purity	% Recovery	Number of naïve CD8 ⁺ T cells recovered from 2.5 x 10 ⁷ starting cells
EasySep™	8	1.0 - 11.2	89.3 ±2.6	64.2 ±25.1	1.0 x 10 ⁵ - 2.2 x 10 ⁶
RoboSep™	4	1.0 - 9.9	89.0 ±5.5	62.7 ±12.5	3.3 x 10 ⁵ - 2.6 x 10 ⁶

Purities determined by flow cytometry. Naïve CD8⁺ T cells are defined as CD8⁺CD45RA⁺CCR7⁺ and CD45RO⁻CD56⁻CD57⁻. Values are expressed as means ±1 sd.

FIGURE 2: Typical FACS plots before and after enrichment of naïve CD8⁺ T cells from PBMC

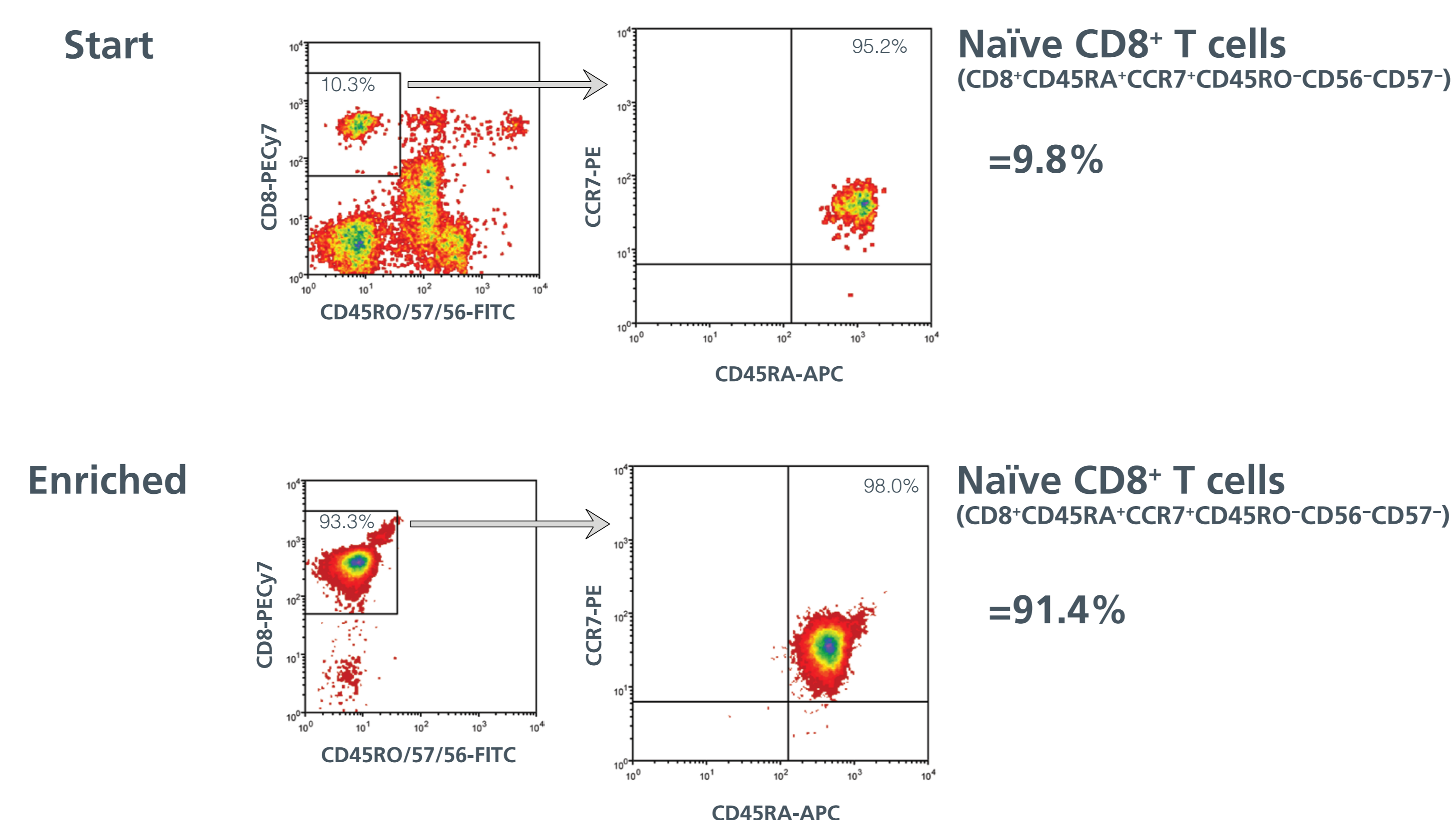
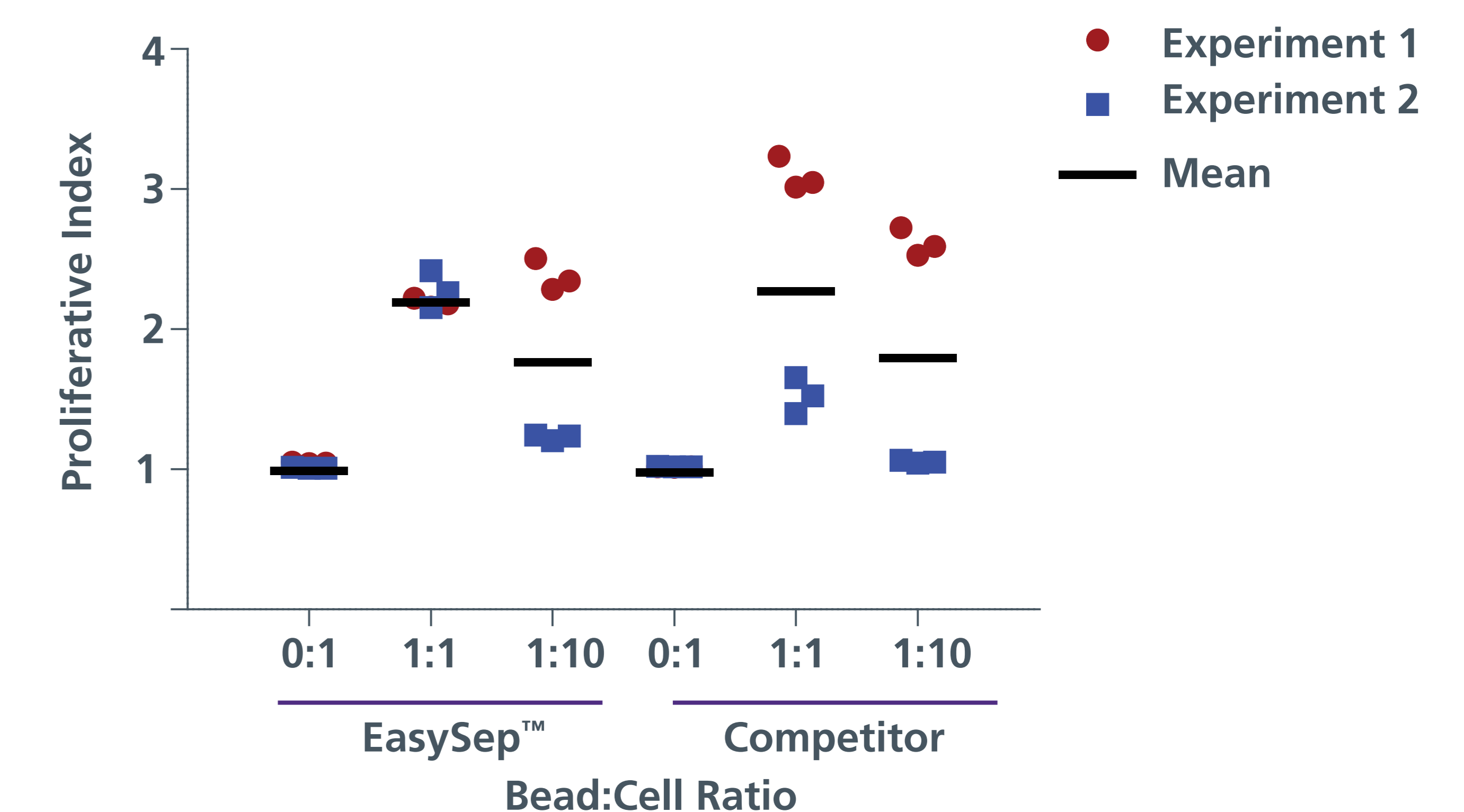


TABLE 3: Comparison of naïve CD8⁺ T cell isolation protocols using EasySep™/RoboSep™ or a column-based competitor kit

	STEMCELL Technologies, Inc.		Competitor
	EasySep™ Naïve CD8 ⁺ T Cell Enrichment Kit (cat #19158)	RoboSep™ Naïve CD8 ⁺ T Cell Enrichment Kit (cat #19158RF)	Column-based human naïve CD8 ⁺ T cell isolation kit
Total time	~50 min	~62 min	~1 hr 45 min
Columns	0	0	2 (3 for high purity)
Centrifugation steps	0	0	4
Isolation method	untouched	untouched	positive selection

FIGURE 3: Comparison of the proliferative response of naïve CD8⁺ T cells isolated by EasySep™ or competitor's kit



The proliferation of naïve CD8⁺ T cells isolated using EasySep™ or a competitor's kit in response to stimulation by anti-CD3/CD28 beads. Proliferation was assessed using a CFSE-based *in vitro* proliferation assay. Anti-CD3/CD28 coated beads were added to freshly isolated naïve CD8⁺ T cells at a final bead to cell ratio of 1:1 or 1:10 and CFSE dye dilution of cells was analyzed on day 4.

Conclusions

- Isolate untouched naïve CD8⁺ T cells from fresh PBMC in under 60 minutes.
- Naïve CD8⁺ T cell isolation can be fully automated with RoboSep™.
- Purities of 89% ±3 and recoveries of 64% ± 25 can be obtained.
- EasySep™ isolated naïve CD8⁺ T cells undergo robust proliferation as assessed using a CFSE-based *in vitro* proliferation assay.
- EasySep™ isolated naïve CD8⁺ T cells are untouched and ideal for functional T cell studies.