A simple and rapid method for the enrichment of mouse naïve CD4⁺ T cells from spleen

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Abstract

Naïve CD4⁺ T cells are a mature subset of CD4⁺ T cells with no previous antigen exposure. The CD62L^{high} CD44^{low} naïve phenotype cells circulate throughout the secondary lymphoid organs where they become activated by foreign antigens presented on MHC class II molecules. Activation is marked by phenotypic changes (down- and up-regulation of CD62L and CD44, respectively), proliferation, and acquisition of effector functions. Current protocols for the isolation of naïve CD4⁺ T cells are time-consuming and require the use of columns. We have developed a one-step, column-free, immunomagnetic cell separation (EasySep™) method for the isolation of naïve CD4⁺ T cells from single-cell suspensions of splenocytes. Non-CD4⁺ T cells, T regulatory cells, and memory CD4⁺ T cells are targeted for depletion using biotinylated antibodies cross-linked to streptavidin-coated magnetic particles. The labeled cells are separated using an EasySep™ magnet and the desired naïve CD4⁺ T cell fraction is poured off. The entire protocol is performed in 15 minutes and can be fully automated using RoboSep™. The average purities and recoveries of CD4⁺CD62L^{high} CD44^{low} cells are 93.1 ± 2.1% and 28.8 ± 7.6%, respectively (n=22). This new method will be invaluable to researchers studying T cell differentiation, signaling pathways, and immune response to infectious disease.

Methods

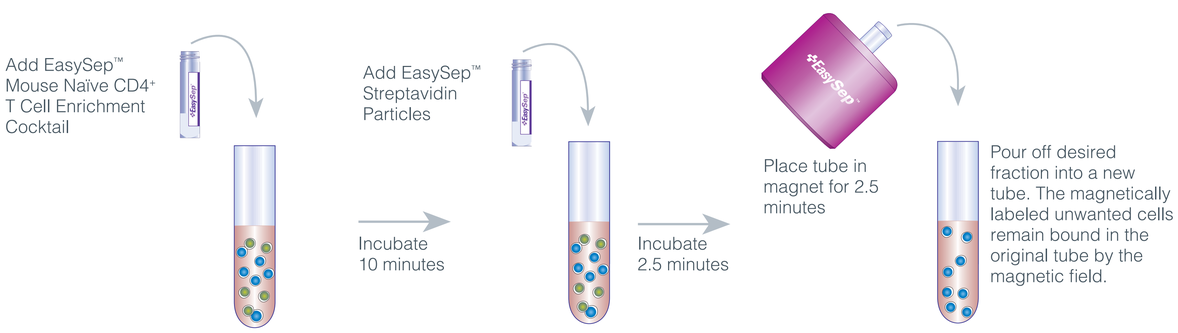
Preparation of Starting Cell Suspension

To prepare a single-cell suspension, spleens were disrupted in phosphate buffered saline (PBS) + 2% fetal bovine serum (FBS). The cells were centrifuged at 300 x *g* for 10 minutes and resuspended at 1x10^8 cells per ml in PBS + 2% FBS with 5% normal rat serum.

EasySep™ Labeling of Mouse Cells

Unwanted cells, including CD4⁻ cells, T regulatory cells, and CD44^{high} CD4⁺ T cells are specifically labeled with biotinylated antibodies cross-linked to steptavidin coated magnetic particles. The magnetically labeled cells are then separated from unlabeled cells using an EasySep™ magnet (**Figure 1**), and the desired naïve CD4⁺ T cell fraction is poured off.

FIGURE 1: EasySep™ procedure for column-free enrichment of naïve CD4⁺ T cells from mouse splenocytes



This procedure can be fully automated using RoboSep™.

Purity Assessment

EasySep™-isolated naïve CD4⁺ T cells were assessed by flow cytometry after staining with CD44 FITC, CD62L PE, CD4 APC, and 7-AAD. Naïve CD4⁺ T cells are CD4⁺CD62L^{high}CD44^{low} (**Figure 2**).

Results

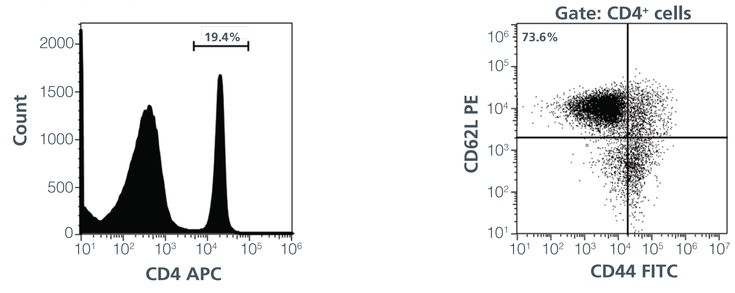
TABLE 1: Purity and recovery of naïve CD4⁺ T cells enriched from mouse splenocytes by EasySep™ or RoboSep™

Method	n	% Purity	% Recovery
EasySep™	16	93.8 ± 1.6	29.6 ± 7.8
RoboSep™	6	91.2 ± 2.4	26.8 ± 7.5

Purities were determined by flow cytometry. All samples gated on viable (7-AAD⁻) cells. Naïve CD4⁺ T cells are defined as CD4⁺CD62L^{high}CD44^{low}. Values are expressed as means ± 1 SD.

FIGURE 2: Flow cytometric assessment of naïve CD4⁺ T cells before and after enrichment using EasySep™

Start: 14.3% CD4⁺CD62L^{high}CD44^{low} viable cells



Enriched: 94.7% CD4⁺CD62L^{high}CD44^{low} viable cells

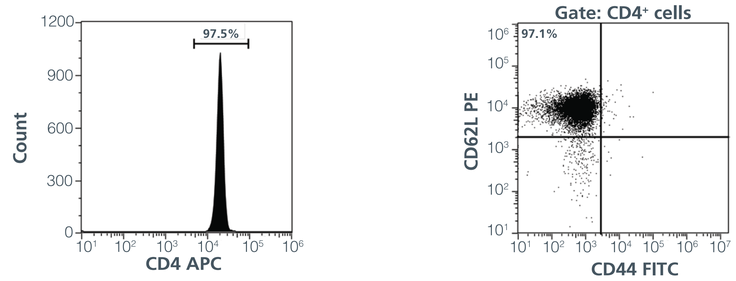


TABLE 2: Comparison of naïve CD4⁺ T cell isolation protocols using EasySep™/RoboSep™ or the column-based competitor kit

	EasySep™	RoboSep™	Competitor
Total time	15 min	22 min	1 hr 50 min
Columns	0	0	2
Centrifugations	0	0	4
Isolation method	untouched	untouched	positive selection

Conclusions

- **Isolate naïve CD4⁺ T cells from mouse splenocytes in 15 minutes.**
- **Naïve CD4⁺ T cell isolation can be fully automated with RoboSep™.**
- **Average purities and recoveries for naïve CD4⁺ T cell enrichments are 93.1% ± 2.1% and 28.8% ± 7.6%, respectively.**