Isolation of highly purified mouse naïve CD4+ T cells in 15 minutes

Vesna Posarac, Armon A. Molavi, Karina L. McQueen, and Terry E. Thomas

STEMCELL Technologies Inc., Vancouver, Canada

Abstract

Naïve CD4+ T cells circulate throughout the secondary lymphoid organs where they become activated by foreign antigens. The pattern of signals present during antigen exposure determines the pathway of naïve CD4+ T cell differentiation into one of at least four distinct CD4+ T cell subsets. Many studies of CD4+ T cell function and differentiation require highly purified naïve CD4+ T cells. To date, this has been achieved by lengthy protocols or flow-based cell sorting. We have developed a rapid one-step immunomagnetic cell separation (EasySep™) method for the isolation of naïve CD4+ T cells from single-cell suspensions of splenocytes. Using our new Streptavidin RapidSphere™ technology, 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 of CD4+CD62L^{high}CD44^{low} cells are 90.8 ± 2.9% (n = 8). The purified naïve CD4+ T cells are fully functional and ready for use in downstream applications as determined by cytokine production assays.

Conclusion

- Isolate naïve CD4+ T cells from mouse splenocytes in 15 minutes
- Naïve CD4+ T cell isolation can be fully automated with RoboSep™
- Average purities for naïve CD4+ T cell isolations are 90.8 ± 2.9%
- Starting with 1 x 10^8 cells, naïve CD4+ T cell recoveries range from 3.9 – 8.8 x 10^6
- Isolated cells are functional and ready for downstream applications as assessed by intracellular cytokine secretion assays

Methods

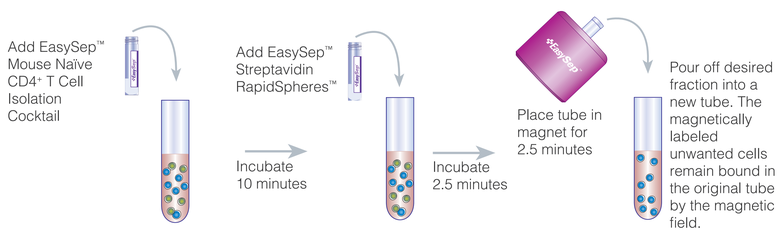
Preparation of Starting Cell Suspension

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 1 x 10^8 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 streptavidin-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 isolation 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

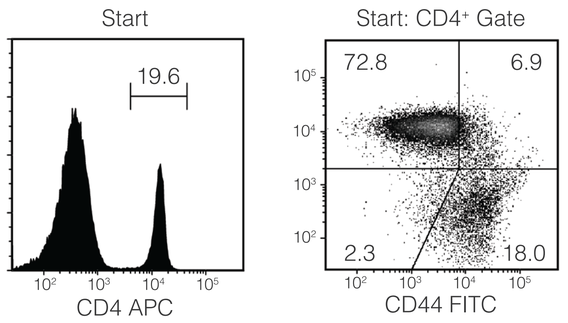
TABLE 1: Purity and recovery of mouse naïve CD4+ T cells isolated from mouse splenocytes by manual EasySep™ or RoboSep™

n	% Start	% Purity of Isolated Cells	Recovery Range Starting with 1 x 10^8 nucleated splenocytes
8	12.6 ± 1.9	90.8 ± 2.9	3.9 – 8.8 x 10^6

Purities determined by flow cytometry. Values are expressed as means ± SD.

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

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



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

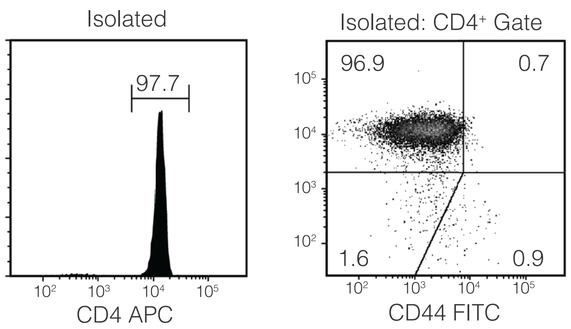
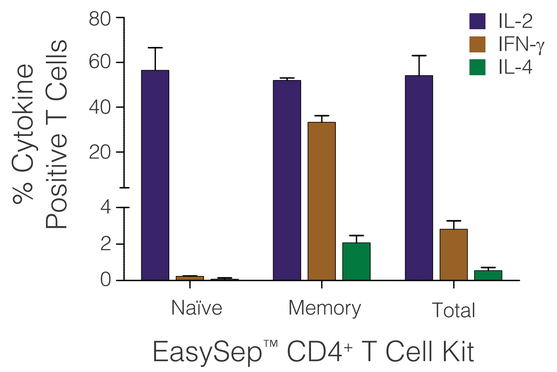


FIGURE 3: Intracellular cytokine secretion assays show cells isolated with the EasySep™ Naïve CD4+ T Cell Kit do not produce effector T cell cytokines upon stimulation



Naïve CD4+ T cells, memory CD4+ T cells, and total CD4+ T cells were isolated by EasySep™, plated in RPMI medium and stimulated with PMA/ionomycin for 6 hours with BFA added during the final 2 hours of culture. Intracellular IL-2, IFN-γ and IL-4 expression in T cells was assessed by flow cytometry. Means of two independent experiments ± SD are shown.

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