Rapid Isolation of CD4$^+$CD25$^+$ Cells from Whole Blood

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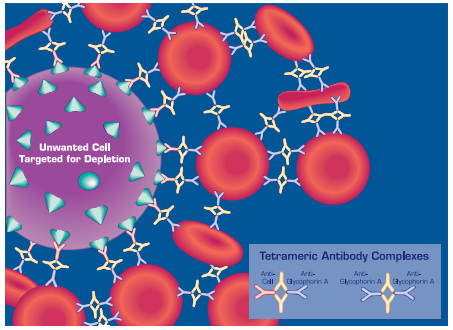
Introduction

The role of CD4$^+$CD25$^+$ regulatory T cells in immunosuppression, maintenance of peripheral tolerance and prevention of autoimmune disorder induction is the subject of an increasing number of clinical and research investigations. Isolation of these cells from whole blood typically involves a red cell removal step followed by time-consuming multi-step immunoselection procedures with low cell recovery. The CD4$^+$CD25$^+$ T cell enrichment method presented here combines two recently developed techniques: an immuno-rosette based density separation method (RosetteSep™) to deplete CD4$^-$ cells followed by an immuno-magnetic positive selection for CD25$^+$ cells using EasySep™. EasySep™ is a non-column based separation system that uses FACS-compatible magnetic nanoparticles. With these two methods, CD4$^+$CD25$^+$ cells can be isolated from whole blood in less than two hours.

Figure 1. Schematic Drawing of RosetteSep™ Immuno-rosetting of Human Cells



Method

Whole blood is incubated with RosetteSep™ CD4$^+$ enrichment cocktail that contains Tetrameric Antibody Complexes (TAC) that crosslink unwanted cells to red blood cells (RBCs), forming immuno-rosettes (Figure 1). Blood is then layered onto Ficoll-Paque® and centrifuged. The unwanted (rosetted) cells pellet along with the free RBCs to the bottom of the tube. CD4$^+$ T cells are collected as an enriched population at the interface between the plasma and the Ficoll-Paque® (Figure 2).

The enriched CD4$^+$T cells are then incubated with bi-specific (anti-CD25 x anti-dextran) TAC followed by dextran-coated magnetic nanoparticles (Figure 3). Cells are then suspended in a standard 5 mL tube that is placed in the EasySep™ magnet. Magnetically labeled CD25$^+$ cells move to the tube wall and after 5 minutes the supernatant is poured off. The tube is removed from the magnet and the captured cells are resuspended. The separation procedure is then repeated twice to improve the purity of the CD4$^+$CD25$^+$ retained fraction, for a total of three separations (Figure 4).

Figure 2. Procedure for RosetteSep™ CD4$^+$ T Cell Enrichment

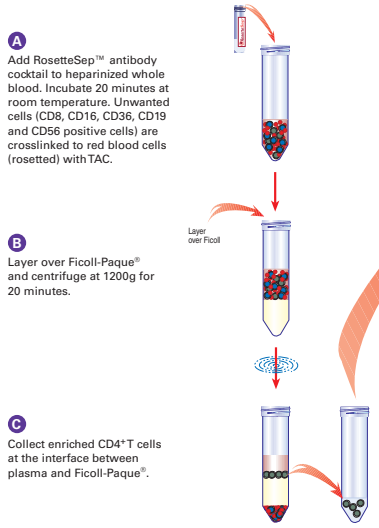


Figure 3. Schematic Drawing of EasySep™ Magnetic Labeling of Human Cells

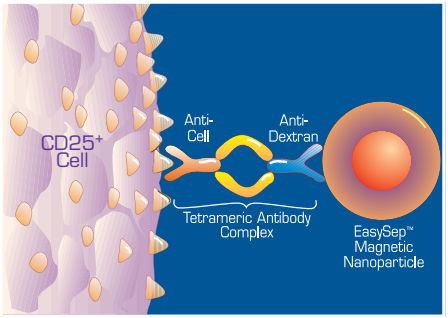


Figure 4. Procedure for EasySep™ CD25 Positive Cell Selection

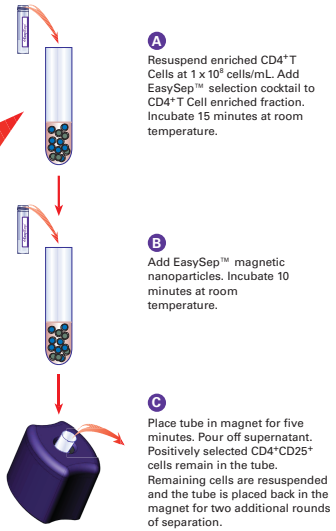
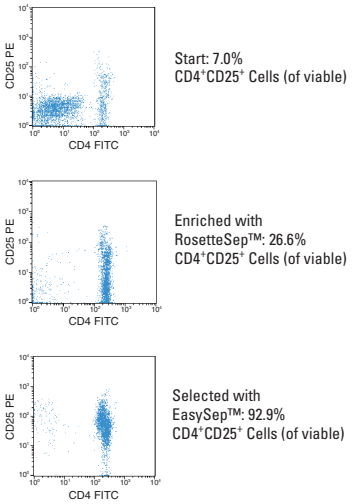


Figure 5. FACS Profiles from a Typical CD4$^+$CD25$^+$ T Cell Enrichment. The dot plots below show viable cell events gated based on PI exclusion.



Results

Table 1. Purity, Cell Yield and Recovery of CD4$^+$CD25$^+$ T Cells. CD4$^+$ T cells were first enriched using RosetteSep™ (85±4% purity and 59±15% recovery). CD25$^+$ cells were then selected using EasySep™. Purity is reported as a percentage of viable (PI negative) cells. Results are expressed as mean ± 1 sd; n=5.

Sample	CD4$^+$CD25$^+$ Purity	Yield of CD4$^+$CD25$^+$ Cells per mL Whole Blood	% Recovery of CD4$^+$CD25$^+$ Cells from Whole Blood
Start	5.6 ± 2.2	$3.6 \times 10^5 \pm 1.3 \times 10^5$	100
After CD4$^+$ T Cell Enrichment with RosetteSep™	35 ± 12	$2.0 \times 10^5 \pm 0.4 \times 10^5$	61 ± 21
After CD4$^+$CD25$^+$ Selection with EasySep™	93 ± 3	$7.2 \times 10^4 \pm 1.4 \times 10^4$	22 ± 6

Conclusions

- Simple method: no columns.
- Rapid: CD4$^+$CD25$^+$ cells are directly isolated from whole blood in less than two hours.
- No particle removal step necessary.
- High CD4$^+$CD25$^+$ purity.
- Cells are recovered in a small volume and are immediately ready for further use.

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