Abstract #1067620

Column-free Isolation of Untouched Human Regulatory T cells in 45 Minutes

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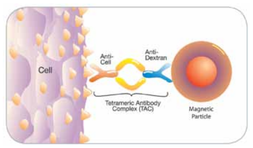
Introduction

Regulatory T cells (Tregs) are a specialized subset of lymphocytes that play a critical role in maintaining immune homeostasis and peripheral tolerance to self antigens. The isolation of highly purified and functional Tregs is essential for advancing this exciting field of research. FOXP3 remains the best marker for the identification of bona fide Tregs, but its intracellular localization currently precludes its use for the isolation of viable human Tregs. To overcome these technical difficulties, researchers have exploited the expression of other surface markers, either alone or in combination, in order to isolate distinct Treg populations. Typically, the expression of CD127 (IL-7 receptor alpha chain) inversely correlates with FOXP3 expression, while CD49d, the alpha-chain of the integrin VLA-4, is expressed on the majority of pro-inflammatory effector cells but is absent on Tregs. Accordingly, removal of CD127^{high} cells from the CD4$^+$ T cell population enriches for a subset of Tregs with high levels of FOXP3 expression. Additional depletion of CD49d expressing cells, further removes contaminating IFN-gamma and IL-17 secreting cells. Together this strategy permits selective isolation of highly enriched Tregs. Using the combination of CD127 and CD49d, we have developed a new kit for the isolation of untouched Tregs from human peripheral blood mononuclear cells (PBMCs) without the need for an additional CD25 positive selection step. The entire isolation procedure starting with PBMCs can be completed in just 45 minutes. Treg enrichment is achieved by antibody mediated crosslinking of unwanted cells to magnetic particles allowing their removal by column-free magnetic separation. Mean CD4$^+$ T cell purity is 92.6% +/- 3.6% of which 83.7% +/- 4.9% express high levels of FOXP3 (n=8). This new product will add to the array of powerful yet convenient tools available for the isolation of functional highly purified human Tregs.

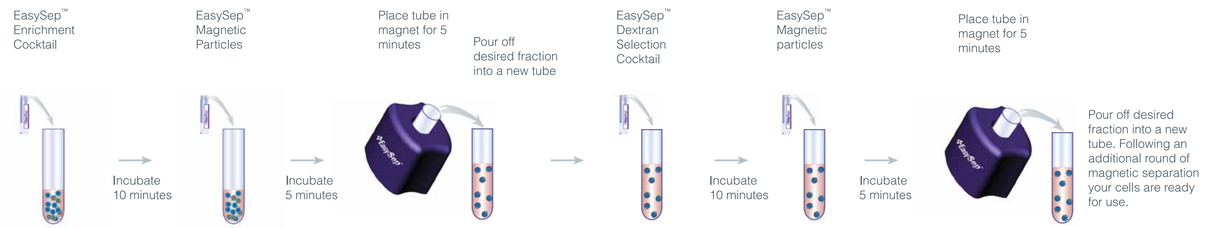
Methods

FIGURE 1: EasySep™ labeling of cells



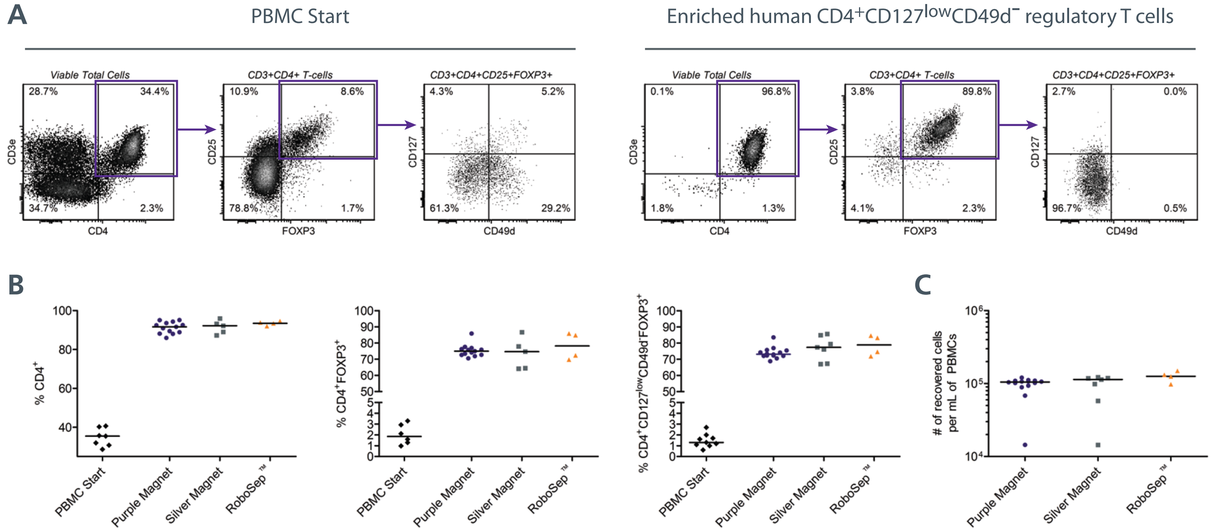
Cells are targeted for selection or depletion using monoclonal antibodies directed against specific cell surface antigens. These labeled cells are then crosslinked to EasySep™ magnetic particles using tetrameric antibody complexes (TAC).

FIGURE 2: EasySep™ protocol for the isolation of untouched human regulatory T cells from PBMCs



Results

FIGURE 3: Phenotypic assessment of EasySep™ and RoboSep™ enriched human CD4$^+$CD127^{low}CD49d$^-$ regulatory T cells



Untouched human CD4$^+$CD127^{low}CD49d$^-$ Tregs isolated using EasySep™ and RoboSep™. A) Purity assessment of Tregs based on viable CD3, CD4, CD25, FOXP3, CD127 and CD49d is shown for a PBMC starting sample and EasySep™ enriched human CD4$^+$CD127^{low}CD49d$^-$ Tregs. B) Purity ranges of PBMC start samples and human CD4$^+$CD127^{low}CD49d$^-$ Tregs enriched using the purple and silver EasySep™ magnets and RoboSep™. C) Total cell recovery numbers from 1x10^8 PBMCs using EasySep™ and RoboSep™. STEMCELL Catalog #: 19232 or 19232RF.

Conclusions

- Untouched human CD4$^+$CD127^{low}CD49d$^-$ regulatory T-cells can be isolated from PBMCs in 45 minutes
- Purities of up to 86% CD3$^+$CD4$^+$CD25$^+$FOXP3$^+$CD127^{low}CD49d$^-$ regulatory T-cells can be achieved
- Highly enriched human regulatory T cells can be rapidly isolated from PBMCs using the manual EasySep™ procedure or the fully automated RoboSep™ cell separator

1. Seddiki, N., *et al.* (2006). JEM. 203: 1693 - 1700.
2. Liu, W. *et al.* (2006). JEM. 203: 1701 - 1711.
3. Kleinewietfeld, M., *et al.* (2009). Blood. 113: 827 - 836.