

Rapid and Versatile Methods for the Column-Free Isolation of Highly Purified and Functional Human Regulatory T Cells



Andy I. Kokaji¹, Lewis Cox², Neil MacDonald¹, Maureen Fairhurst¹, and Terry E. Thomas¹

¹STEMCELL Technologies Inc., Vancouver, BC, Canada

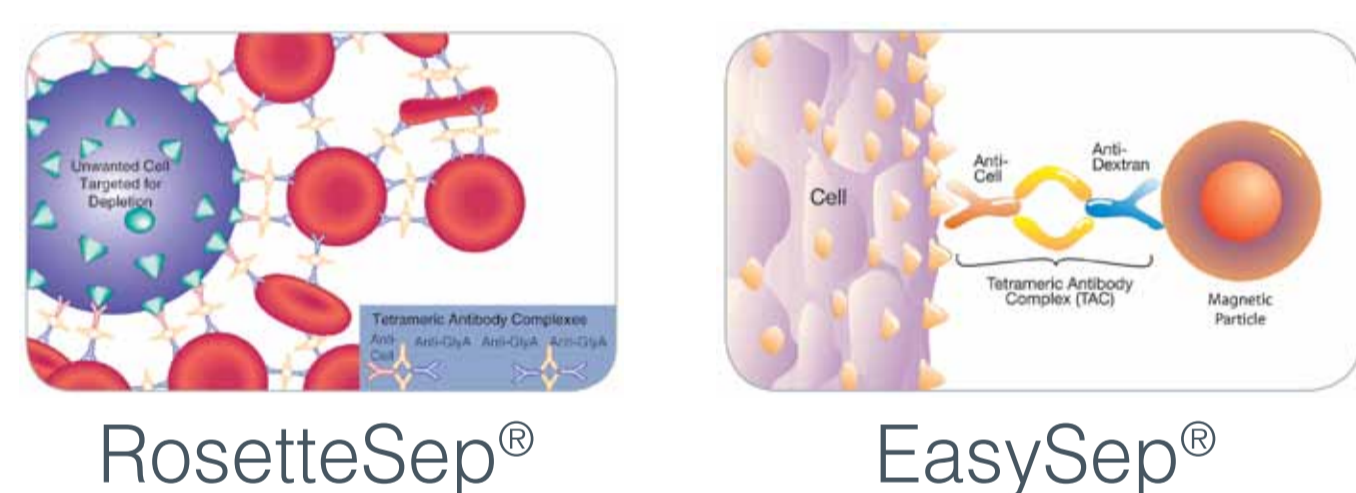
²STEMCELL Technologies Inc., Melbourne, Victoria, Australia

Abstract

Regulatory T cells (Tregs) are a specialized subset of T cells that plays a key role in immune regulation. Harnessing the suppressive function of Tregs is a major area of interest as they hold great potential for the treatment of autoimmune disorders. STEMCELL Technologies has developed a full range of products for the rapid and efficient isolation of highly functional Tregs from virtually any peripheral blood sample using their unique cell separation platforms RosetteSep[®] (immunodensity cell separation) and EasySep[®] (immunomagnetic cell separation). Treg pre-enrichment is achieved by antibody-mediated crosslinking of unwanted cells to either red blood cells (RosetteSep[®]) or magnetic particles (EasySep[®]), allowing their removal by Ficoll centrifugation or magnetic separation, respectively. RosetteSep[®] or EasySep[®] pre-enriched Treg populations consist of CD4⁺, CD4⁺CD127^{low} or CD4⁺CD127^{low}CD49d⁻ T cells. Pre-enriched Tregs can be further purified using EasySep[®] positive selection to isolate Tregs expressing high levels of cell surface CD25. Purities of 85% ±10% CD4⁺CD25^{high}FOXP3⁺ human Tregs can be achieved depending on the Treg population. From start to finish, Treg isolations can be completed in less than 3 hours. Purified Tregs display suppressive activity both immediately upon isolation and after expansion for 14 days.

Methods

FIGURE 1: RosetteSep[®] and EasySep[®] Cell Separation



Cells are targeted for selection or depletion using monoclonal antibodies directed against specific cell surface antigens. These labeled cells are then cross-linked to either red blood cells (RosetteSep[®]) or EasySep[®] magnetic particles using tetrameric antibody complexes (TAC).

FIGURE 2: Time Savings Using RosetteSep[®] and EasySep[®]

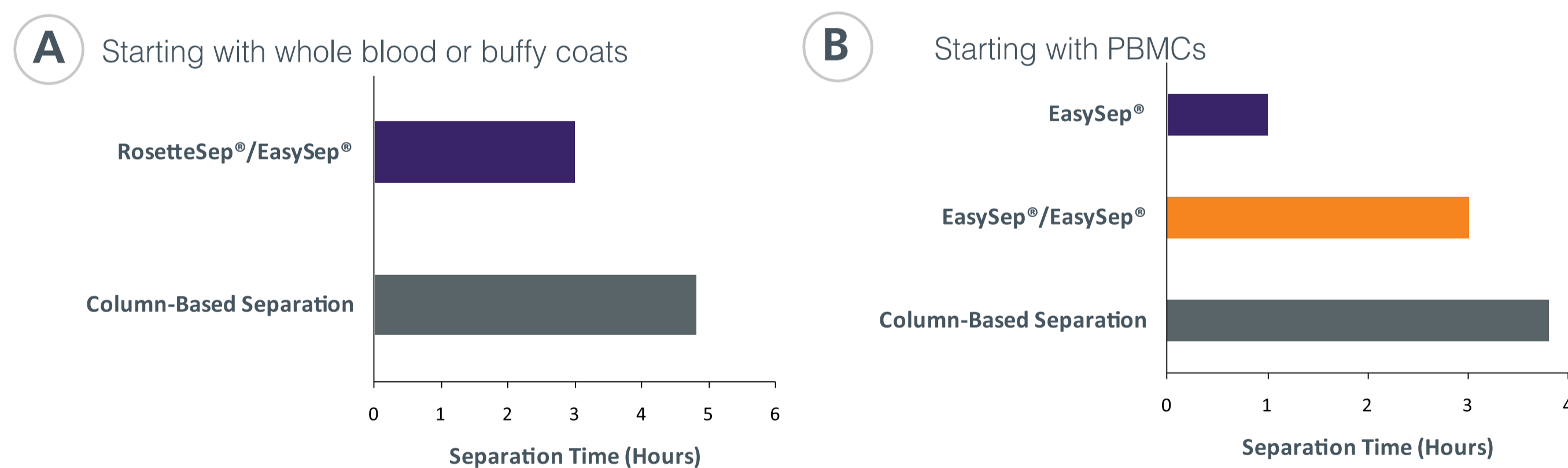
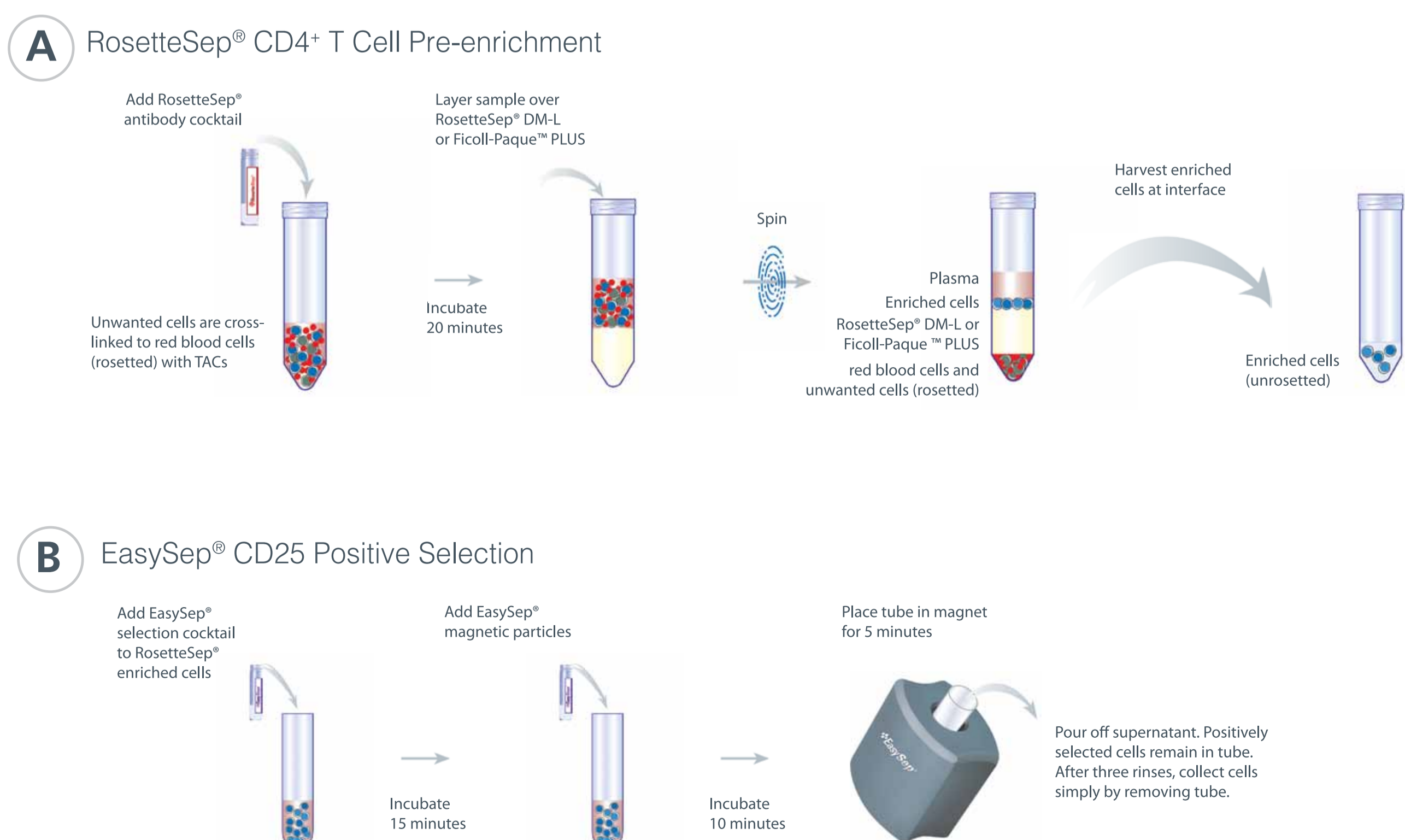
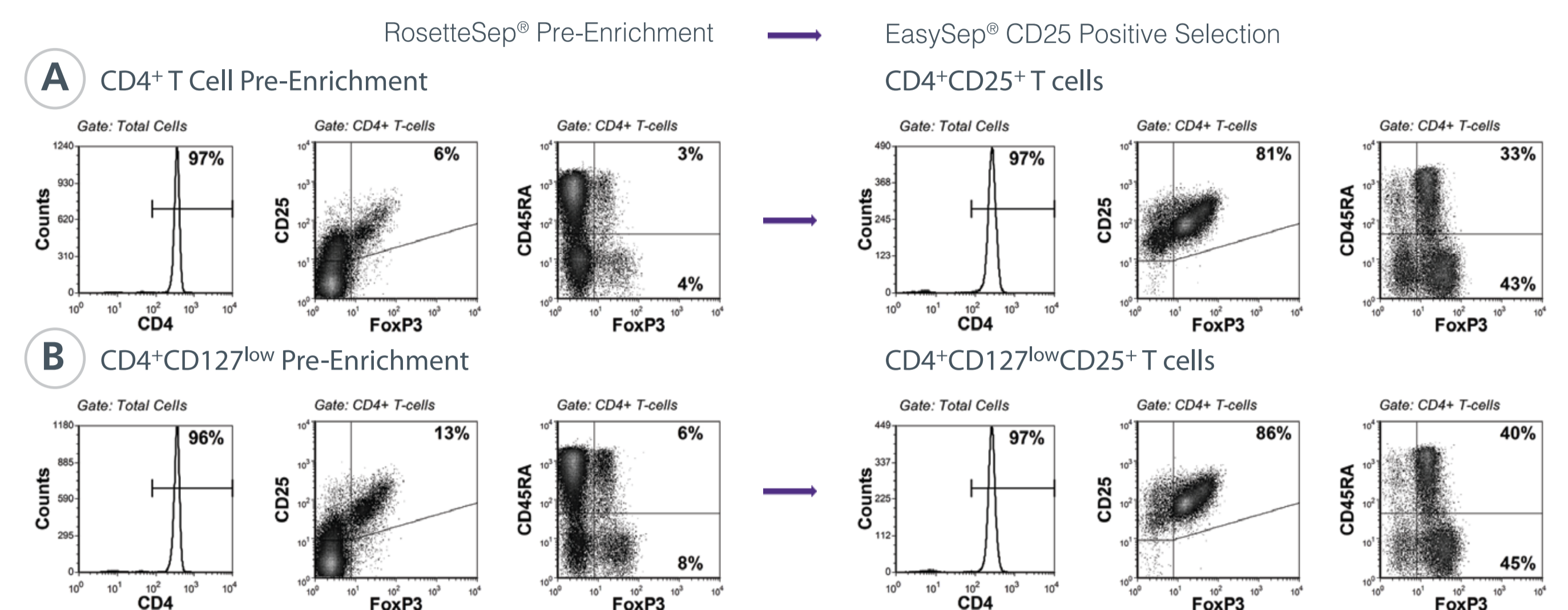


FIGURE 3: Isolation of Human Tregs from Whole Blood or Buffy Coats



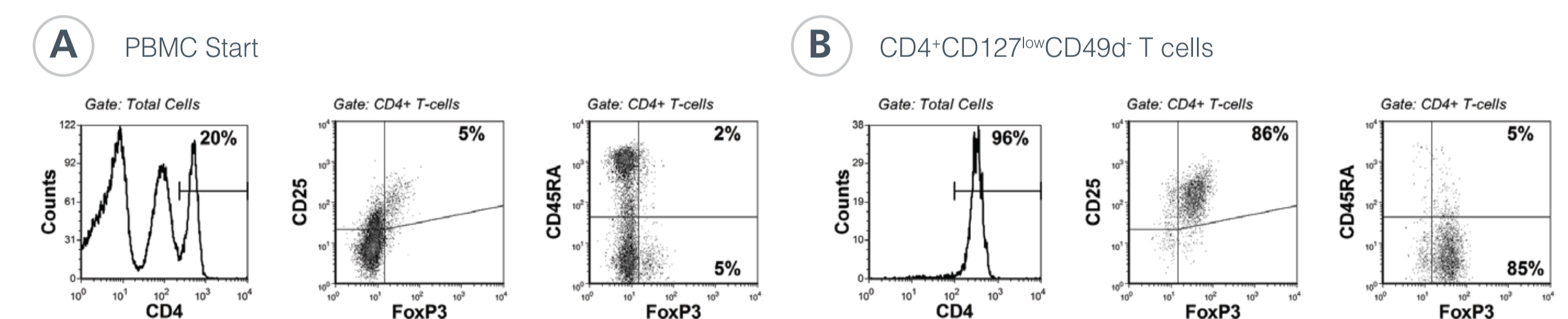
Results

FIGURE 4: Purity and Phenotype of Purified Human Tregs



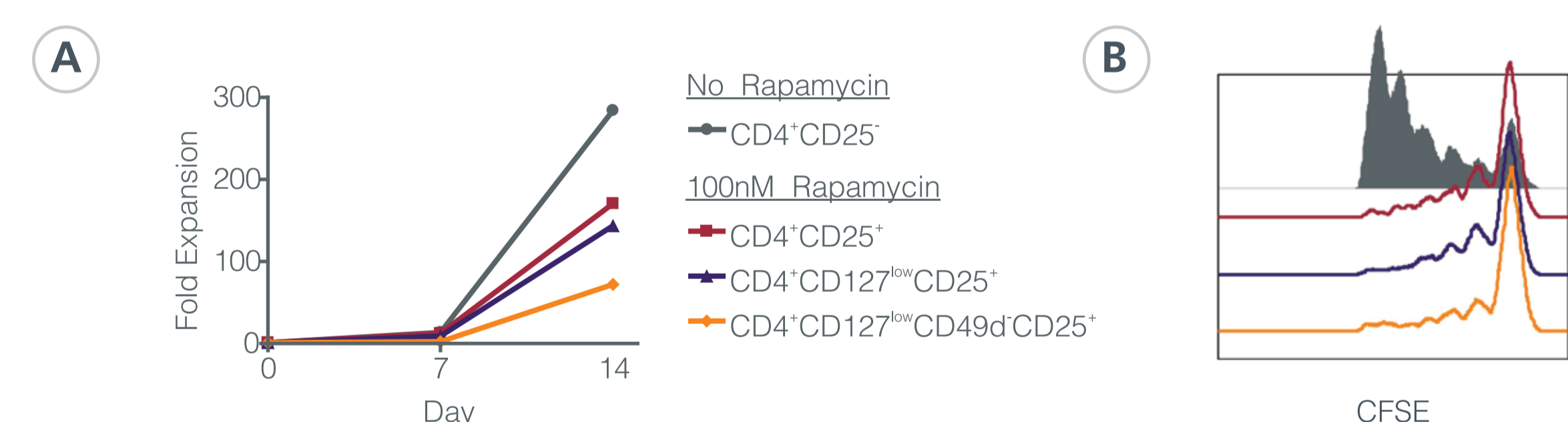
Human Tregs were isolated from whole blood using RosetteSep[®] followed by EasySep[®] CD25 positive selection. Purity and phenotype of Tregs based on CD4, CD25, FoxP3, and CD45RA is shown using RosetteSep[®] A) CD4⁺, B) CD4⁺CD127^{low} and C) CD4⁺CD127^{low}CD49d⁻ pre-enrichment followed by EasySep[®] CD25 positive selection. STEMCELL Catalog Number A) 15862 and B) 15861.

FIGURE 5: Untouched Human Tregs Isolated using EasySep[®]



Untouched human CD4⁺CD127^{low}CD49d⁻ Tregs were isolated using EasySep[®]. Purity and phenotype of Tregs based on CD4, CD25, FoxP3, and CD45RA is shown for A) PBMC starting sample and B) CD4⁺CD127^{low}CD49d⁻ Tregs following EasySep[®] negative enrichment (STEMCELL Catalog #19232).

FIGURE 6: Isolated Human Tregs can be Expanded *in vitro* while Maintaining their Functionality



The ability of *ex vivo* expanded human Tregs to suppress anti-CD3/CD28 bead induced T cell proliferation was assessed using a CFSE based *in vitro* suppression assay. A) Purified Tregs were expanded for 14 days in the presence of 100nM rapamycin, 500U/mL IL-2 and anti-CD3/CD28 coated beads. B) Suppression assays were performed using a ratio of 1:2 expanded Tregs to autologous CFSE labeled CD4⁺CD25⁻ T cells in the presence of anti-CD3/CD28 beads for 4 days.

Conclusions

- Human regulatory T cells can be isolated from whole blood in less than three hours using a combination of RosetteSep[®] and EasySep[®].
- Untouched CD4⁺CD127^{low}CD49d⁻ Tregs can be isolated from PBMCs in one hour using EasySep[®] or RoboSep[®].
- Human Tregs isolated using RosetteSep[®] and EasySep[®] can suppress T cell proliferation and can be expanded *in vitro* while maintaining their functionality.