Column-free methods for the rapid isolation of highly purified, functional and expandable human regulatory T cells

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Abstract

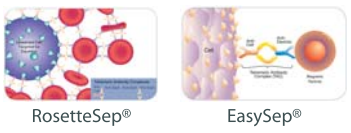
Regulatory T cells (Tregs) are a specialized subset of T cells that play a key role in peripheral tolerance and immune regulation. Therefore, harnessing the suppressive function of Tregs is a major area of interest as they hold great potential for the treatment of autoimmune disorders. In addition, these cells are of extreme interest to researchers investigating transplantation tolerance, cancer and vaccine development. Unlike their mouse counterparts, human Tregs appear to display a heterogeneous cell surface phenotype and regulatory capacity. To complicate matters further, human peripheral blood Tregs comprise only a small fraction of total $CD4^+$ T cells and therefore must be highly enriched for their regulatory function to be detectable *in vitro*. Since Tregs lack a unique cell surface marker to distinguish them from activated T cells, it is difficult to isolate highly purified Tregs in order to evaluate their function and therapeutic potential. With unique cell separation platforms, STEMCELL has developed a full range of products for the rapid and efficient isolation of highly functional Tregs from any normal peripheral blood sample. RosetteSep® is an antibody mediated buoyant density centrifugation method used to isolate untouched subsets of PBMCs specifically from whole blood or buffy coat samples. EasySep® is an immunomagnetic cell separation method used to isolate cells from fresh or previously frozen PBMCs. 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 consisting of $CD4^+$, $CD4^+CD127^{low}$ or $CD4^+CD127^{low}CD49d^-$ T cells can then be further purified using EasySep® positive selection to isolate Tregs expressing high levels of cell surface CD25. Purities of up to 90% $CD4^+CD25^{high}FoxP3^+$ human Tregs can be achieved depending on the desired Treg population. From start to finish, Treg isolations can be completed in less than 3 hours and they can be used immediately in downstream assays or expanded *in vitro* while maintaining their functionality.

Conclusions

- Human regulatory T cells can be isolated from whole blood in less than 3 hours using a combination of RosetteSep® and EasySep®
- Highly enriched human regulatory T cells can be rapidly isolated from PBMCs using the manual EasySep® or the fully automated RoboSep® system
- Untouched $CD4^+CD127^{low}CD49d^-$ Tregs can be isolated from PBMCs in one hour using EasySep® or RoboSep®
- Pre-enrichment of Tregs using RosetteSep® or EasySep® can significantly reduce flow sorting times for isolating human regulatory T cell subpopulations at >95% purity
- Human regulatory T cells isolated using RosetteSep® and EasySep® can suppress T cell proliferation and can be expanded *in vitro* while maintaining their functionality

Methods

FIGURE 1: RosetteSep® and 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 either red blood cells (RosetteSep®) or EasySep® magnetic particles using tetrameric antibody complexes (TAC).

FIGURE 2: Significant time savings using RosetteSep® and EasySep®

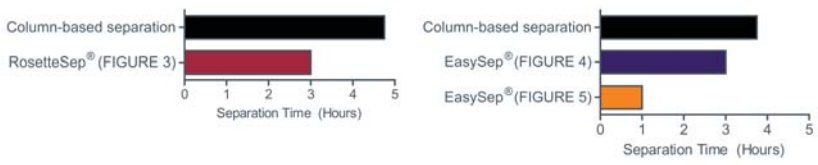
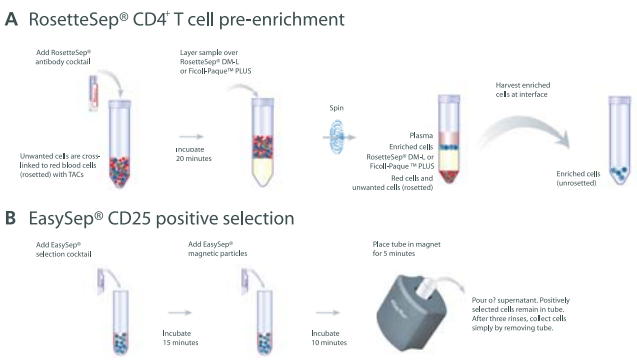
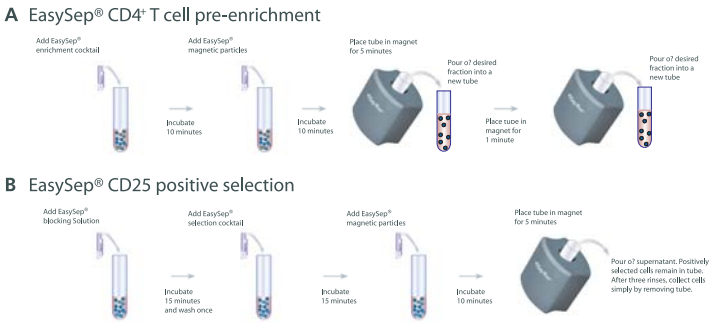


FIGURE 3: Human regulatory T cell isolation starting with whole blood or buffy coats using RosetteSep® and EasySep®



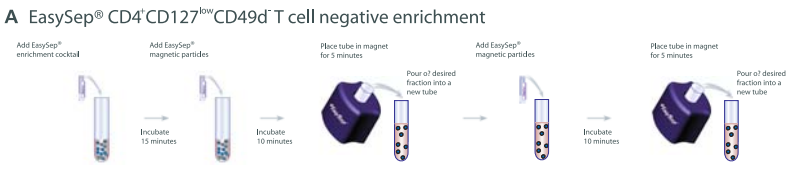
Representative purity and functional data of Tregs isolated from whole blood using RosetteSep® and EasySep® is shown in Figure 7 and Figure 10, respectively.

FIGURE 4: Human regulatory T cell isolation starting with PBMCs using EasySep®



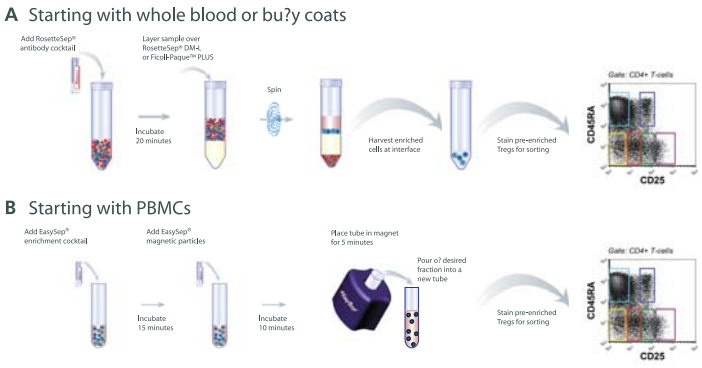
Representative purity data of EasySep® isolated $CD4^+CD25^+$ T cells is shown in Figure 8.

FIGURE 5: Untouched human regulatory T cells starting with PBMCs using EasySep®



Representative purity data of EasySep® isolated $CD4^+CD127^{low}CD49d^-$ T cells shown in Figure 9.

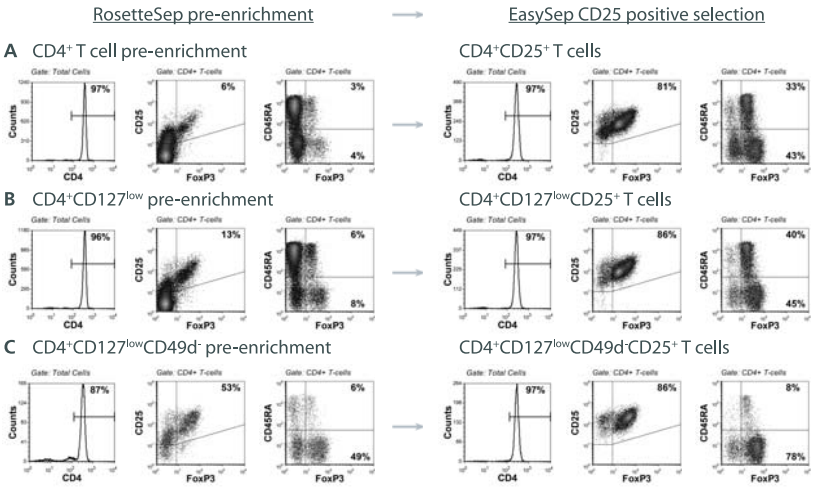
FIGURE 6: Reduce flow sorting time by pre-enriching human regulatory T cells from whole blood, buffy coats or PBMCs



In as little as 90 minutes, human Tregs can be pre-enriched from A) whole blood or buffy coat samples; or B) PBMCs, stained with anti-CD4,-CD25, -CD127 and -CD45RA and subsequently sorted into specific Treg subpopulations.
STEMCELL Catalog #: A) 15361 or B) 19231

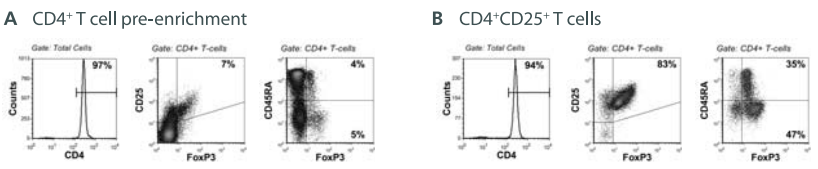
Results

FIGURE 7: Purity and phenotype of human regulatory T cells isolated using RosetteSep® and EasySep®



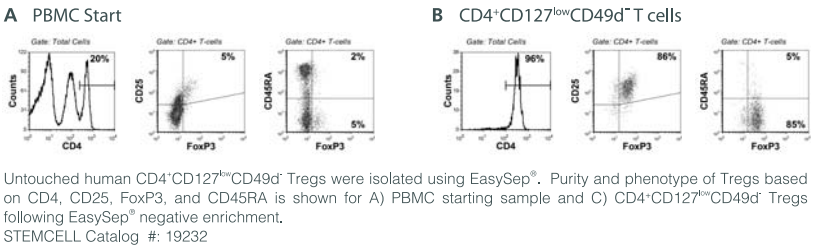
Human Tregs were isolated from whole blood using RosetteSep® followed by EasySep® $CD25^{high}$ positive selection. Purity and phenotype of Tregs based on CD4, CD25, FoxP3, and CD45RA is shown using RosetteSep® A) $CD4^+$, B) $CD4^+CD127^{low}$ and C) $CD4^+CD127^{low}CD49d^-$ pre-enrichment followed by EasySep® $CD25^{high}$ positive selection.
STEMCELL Catalog #: A) 15862, B) 15861 and C) 15864.

FIGURE 8: Purity and phenotype of human regulatory T cells isolated from PBMC using RoboSep®



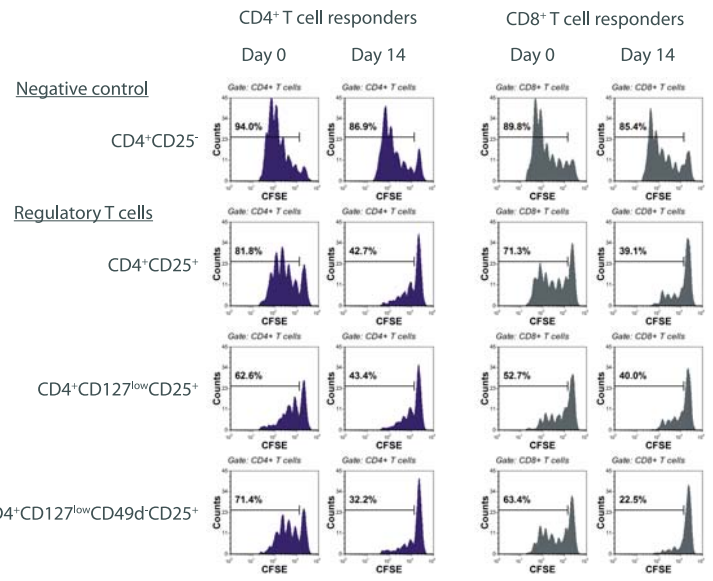
Human $CD4^+CD25^+$ Tregs were isolated using RoboSep®, the fully automated cell separator. RoboSep® uses EasySep® cell separation reagents and performs all labeling and magnetic separation steps for true walk away automation. Purity and phenotype of Tregs based on CD4, CD25, FoxP3, and CD45RA is shown for A) enriched $CD4^+$ T cells and B) $CD4^+CD25^+$ Tregs following RoboSep® $CD25^{high}$ positive selection.
STEMCELL Catalog #18062R

FIGURE 9: Purity and phenotype of untouched human regulatory T cells 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 C) $CD4^+CD127^{low}CD49d^-$ Tregs following EasySep® negative enrichment.
STEMCELL Catalog #: 19232

FIGURE 10: Isolated human regulatory T cells can be expanded *in vitro* while maintaining their functionality



The ability of freshly isolated or expanded human Tregs to suppress anti-CD3/CD28 induced T cell proliferation was assessed using a CFSE based *in vitro* suppression assay. Human Tregs were isolated from whole blood using RosetteSep® followed by EasySep® $CD25^{high}$ positive selection. Tregs were either used immediately (day 0) or expanded for 14 days in the presence of 100nM rapamycin, 500U/mL IL-2 and anti-CD3/CD28 coated beads. As a control, $CD4^+CD25^-$ T cells were expanded in the absence of rapamycin. Suppression assays were performed using a ratio of 1:1 Tregs to autologous CFSE labelled PBMCs in the presence of soluble anti-CD3 and -CD28. Following four days of culture, cells were harvested and CFSE dilution was assessed on gated $CD4^+$ and $CD8^+$ T cells.