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. However, the current methods of purifying Tregs are labor-intensive and time-consuming. Here we describe a novel method that allows for the rapid and efficient isolation of highly functional Tregs from whole blood samples. The method is based on an antigen-specific magnetic depletion and positive selection strategy. Briefly, whole blood samples are incubated with anti-CD25 magnetic beads, which bind to the CD25 molecules on Tregs. The mixture is then passed through a magnetic column, allowing the Tregs to be enriched while leaving the other immune cells behind. The remaining immune cells are then depleted using anti-CD4 and anti-CD8 antibodies.

Conclusions
- Human regulatory T cells can be isolated from whole blood in less than 3 hours using a combination of RosetteSep<sup>®</sup> and EasySep<sup>®</sup> technology.
- Highly enriched human regulatory T cells can be rapidly isolated from PBMCs using the manual EasySep<sup>®</sup> or the fully automated RosoEasy<sup>®</sup> system.
- Unfractionated CD4<sup>+</sup>CD25<sup>+</sup> Tregs can be isolated from PBMCs in one hour using EasySep<sup>®</sup> or RosoEasy<sup>®</sup>.
- Pre-enrichment of Tregs using RosetteSep<sup>®</sup> or EasySep<sup>®</sup> can significantly reduce flow sorting time for isolating human regulatory T cells from whole blood.
- Human regulatory T cells isolated using RosetteSep<sup>®</sup> and EasySep<sup>®</sup> can suppress T cell proliferation and can be expanded in vitro while maintaining their functionality.

Methods
**FIGURE 1:** RosetteSep<sup>®</sup> and EasySep<sup>®</sup> labeling of cells

**FIGURE 2:** Significant time savings using RosetteSep<sup>®</sup> and EasySep<sup>®</sup>

A. Starting with whole blood or buffy coats
B. Starting with PBMCs

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

A. RosetteSep<sup>®</sup> iEDT T cell pre-enrichment
B. EasySep<sup>®</sup> CD4<sup>+</sup> position selection

**FIGURE 4:** Human regulatory T cell isolation starting with PBMCs using EasySep<sup>®</sup>

A. EasySep<sup>®</sup> CD4<sup>+</sup> pre-enrichment
B. EasySep<sup>®</sup> CD4<sup>+</sup> position selection

**FIGURE 5:** Untouched human regulatory T cells starting with PBMCs using EasySep<sup>®</sup>

A. EasySep<sup>®</sup> CD4<sup>+</sup>CD127<sup>+</sup>CD25<sup>+</sup> T cell negative enrichment

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

A. Starting with whole blood or buffy coats
B. Starting with PBMCs

Results
**FIGURE 7:** Purity and phenotype of human regulatory T cell isolates using RosetteSep<sup>®</sup> and EasySep<sup>®</sup>

A. CD4<sup>+</sup> T cell pre-enrichment
B. CD4<sup>+</sup>CD25<sup>+</sup> T cell
C. CD4<sup>+</sup>CD127<sup>+</sup>CD25<sup>+</sup> T cell pre-enrichment
D. CD4<sup>+</sup>CD127<sup>+</sup>CD25<sup>+</sup> T cell

**FIGURE 8:** Purity and phenotype of human regulatory T cells isolated from PBMC using RosoEasy<sup>®</sup>

A. CD4<sup>+</sup> T cell pre-enrichment
B. CD4<sup>+</sup>CD25<sup>+</sup> T cell
C. CD4<sup>+</sup>CD127<sup>+</sup>CD25<sup>+</sup> T cell pre-enrichment
D. CD4<sup>+</sup>CD127<sup>+</sup>CD25<sup>+</sup> T cell

**FIGURE 9:** Purity and phenotype of untouched human regulatory T cells isolated using EasySep<sup>®</sup>

A. PBMCs
B. CD4<sup>+</sup>CD127<sup>+</sup>CD25<sup>+</sup> T cell

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

The ability of isolated human regulatory T cells to suppress and expand in vitro upon culture was assessed. Human Tregs were expanded from whole blood using RosoEasy<sup>®</sup> followed by EasySep<sup>®</sup> CD4<sup>+</sup>CD25<sup>+</sup> positive selection. Tregs were then cocultured with non-Treg cells. Proliferation assays were performed using a 5-day pulse of [3H]-thymidine. HLA and TCR expression was confirmed on day 5.