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

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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® and EasySep® (immunodemography cell separation) and EasySep® (immunodemography 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+, or CD4/CD127−/CD49d- T cells. Pre-enriched Tregs can be further purified using EasySep® positive selection to isolate Tregs expressing high levels of cell surface CD25. Purifies of 85% ±10% CD4+CD25+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 biotinylated antibodies are then crosslinked to either red blood cells (RosetteSep®) or EasySep® magnetic particles using tetrameric antibody complexes (TAC).

FIGURE 2: Time Savings Using RosetteSep® and EasySep®

(A) Starting with whole blood oruffy coats

(B) Starting with PBMCs

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

(A) RosetteSep® CD4+ T Cell Pre-enrichment

(B) EasySep® CD25 Positive Selection

Results

FIGURE 4: Purity and Phenotype of Purified Human Tregs

(D) CD4+ CD127+ CD49d- T cell populations consisted of CD4+, CD4+/CD127+, or CD4/CD127−/CD49d- T cells. Pre-enriched Tregs can be further purified using EasySep® positive selection to isolate Tregs expressing high levels of cell surface CD25. Purifies of 85% ±10% CD4+CD25+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.

FIGURE 5: Untouched Human Tregs Isolated using EasySep®

(A) PBMC Start

(B) CD4+CD127+CD49d- T cells

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. Untouched Tregs were expanded for 14 days in the presence of 100nM rapamycin, 1000U/mL IL-2, and anti-CD3/CD28 coated beads. 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+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.