

Isolation of highly purified mouse CD4⁺CD25⁺FoxP3⁺ regulatory T cells in less than 45 minutes

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Summary

Regulatory T cells (Tregs), a subset of lymphocytes, play a key role in maintaining peripheral tolerance, preventing autoimmune diseases and limiting chronic inflammatory diseases. They can be broadly classified into natural or adaptive (induced) Tregs. Natural Tregs (CD4⁺CD25⁺FoxP3⁺) develop in the thymus and migrate to the periphery to help maintain immune homeostasis. Adaptive Tregs can be induced from CD25-negative naïve CD4⁺ T cells in the thymus and acquire CD25 (IL-2R alpha) and FoxP3 expression in the periphery after adequate antigenic stimulation. They are typically induced by chronic allergic inflammation and disease processes, such as autoimmunity. The isolation of highly purified Tregs is essential for advancing research in this field. To date this has been achieved by lengthy protocols or flow-based cell sorting. We have developed a rapid column-free one-step immunomagnetic cell separation method (EasySep™) to isolate Tregs (natural and induced) from mouse splenocytes in less than 45 minutes. CD25⁺ cells are labeled with anti-CD25-PE antibody and bound to magnetic particles by antibody complexes recognizing PE and the dextran coating on the particle. The CD25-PE-labeled cells are then separated using an EasySep™ magnet. The procedure can be automated using RoboSep™. Starting from 2.2 ± 0.4% Tregs, purities of 84 ± 3% (mean ± SD, n = 20) CD4⁺CD25⁺FoxP3⁺ cells can be achieved. This kit provides a new tool to study the mechanisms by which Tregs exert their influence, which has broad implications for the development of cell-based therapies for autoimmune disease and cancer.

Methods

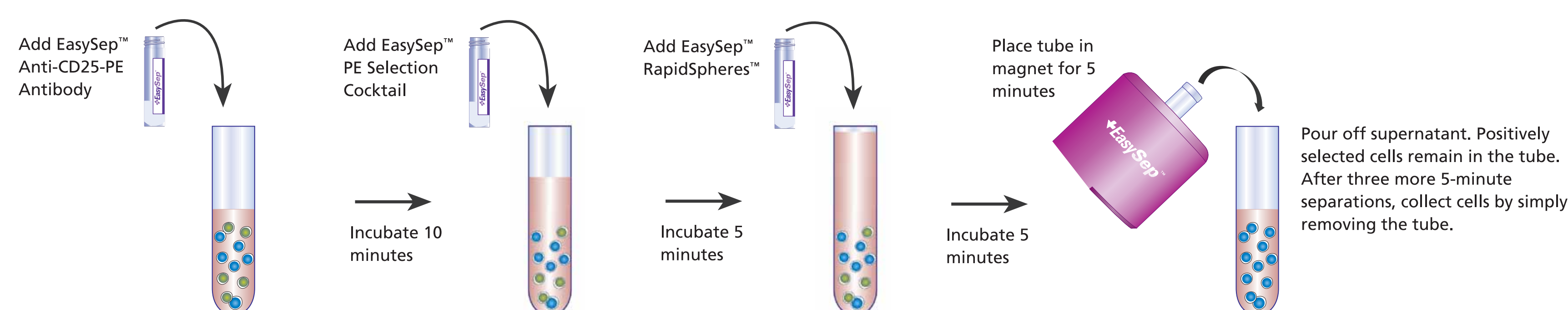
Preparation of the Starting Cell Suspension

To prepare a single-cell suspension, spleens were disrupted in phosphate buffered saline (PBS) + 2% fetal bovine serum (FBS). The cells were centrifuged at 300 x g for 10 minutes and resuspended at 1 x 10⁸ cells/mL in PBS + 2% FBS.

EasySep™ Labeling of Mouse Cells

Regulatory T cells are first labeled with an anti-CD25-PE antibody and are bound to magnetic particles through antibody complexes recognizing PE and the dextran coating on the particle surface. Labeled cells are separated using an EasySep™ magnet without the use of columns. CD25⁺ cells remain in the tube while unwanted cells are poured off.

FIGURE 1: EasySep™ procedure for column-free isolation of CD25⁺ cells from mouse splenocytes



This procedure can be fully automated using RoboSep™.

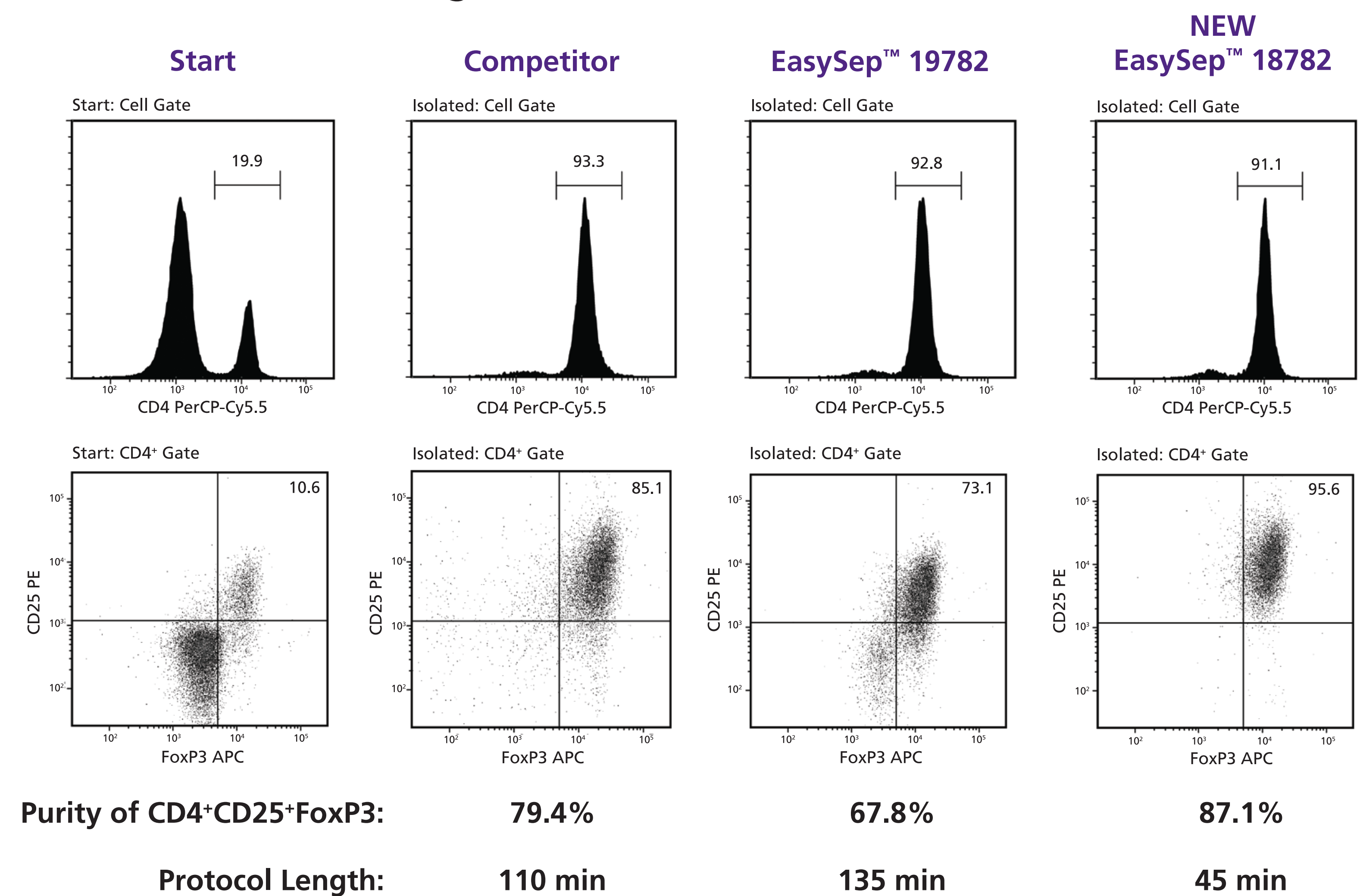
Results

TABLE 1: Purity and recovery of mouse CD25⁺ regulatory T cells isolated from splenocytes using the EasySep™ Mouse CD25⁺ Regulatory T Cell Positive Selection Kit (Catalog #18782)

Method	n	% Start	% Purity of CD4 ⁺ CD25 ⁺ FoxP3 ⁺ Cells	Average Recovery from 1 x 10 ⁸ nucleated cells
EasySep™	14	2.1 ± 0.4	84.7 ± 3.0	8.4 x 10 ⁵
RoboSep™	6		83.5 ± 2.5	3.3 x 10 ⁵

Purities determined by flow cytometry. Values are expressed as mean ± SD.

FIGURE 2: Head-to-head comparison of the new EasySep™ Mouse CD25⁺ Regulatory T Cell Isolation Kit (Catalog #18782) compared to a competitor's kit and the STEMCELL EasySep™ Mouse CD4⁺CD25⁺ Regulatory T Cell Isolation Kit (Catalog #19782)



Both the competitor and EasySep™ 19782 kits use a 2-step isolation strategy where CD4⁺ cells are pre-enriched prior to CD25 positive selection. The new EasySep™ 18782 kit employs a CD25⁺ positive isolation strategy without CD4⁺ T cell pre-enrichment.

TABLE 3: Head-to-head comparison of CD4⁺CD25⁺FoxP3⁺ purity, as well as CD4⁺CD25⁻, B cell, and CD8⁺ T cell contaminants present in the EasySep™ Mouse CD25⁺ Regulatory T Cell Isolation Kit compared to a competitor's isolation kit

n = 5	% Purity of CD4 ⁺ CD25 ⁺ FoxP3 ⁺ Cells	% of CD4 ⁺ CD25 ⁻ Cells	% of CD4 ⁺ CD19 ⁺ Cells	% of CD4 ⁺ CD8 ⁺ Cells
EasySep™ 18782	83.5 ± 4.8	2.1 ± 1.8	4.2 ± 2	0.5 ± 0.4
Competitor	78.9 ± 3.7	8.2 ± 1.3	0.4 ± 0.2	0.1 ± 0.1
p-value (paired t-test)	0.017	0.0063	0.016	0.01

Purities determined by flow cytometry. Values are expressed as mean ± SD.

Conclusions

- Isolate highly purified CD4⁺CD25⁺FoxP3⁺ cells from mouse splenocytes in 45 minutes
- Regulatory T cell isolation can be fully automated with RoboSep™
- Average CD4⁺CD25⁺FoxP3⁺ purities are 84 ± 3%
- Minimal CD8⁺ T cell and CD4⁺CD25⁻ contamination