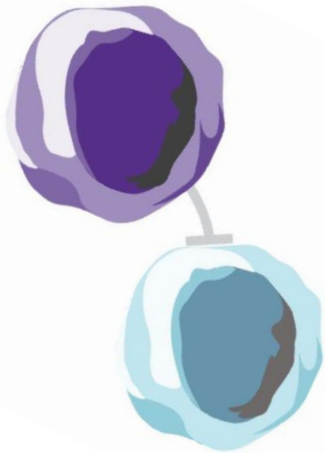


# Fast and Versatile Isolation of Regulatory T Cells

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STEMCELL Technologies



## Research Significance

- Regulatory T cells (Tregs) are involved in:
  - Autoimmunity
  - Immune regulation and homeostasis
  - Tolerance to self antigens
  - Graft acceptance / rejection (i.e. GVHD)
  - Cancer immunotherapy
  - Allergic responses
  - Infectious diseases

# Challenges Associated with Isolating Tregs

## 1. *Human Tregs have a heterogeneous phenotype*

- No single cell surface marker can uniquely identify Tregs
- Human Tregs are CD4<sup>+</sup> and express high levels of CD25
- CD25 and CD45RA expression can delineate functionally different subpopulations of FoxP3<sup>+</sup> human Tregs

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- Contamination by effector cells can be detrimental to experiments

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## 2. *Highly purified Tregs are critical for assay success*

- Contamination by effector cells can be detrimental to experiments

## 3. *Tregs are present at a very low frequency in peripheral blood*

- ~4x10<sup>4</sup> per ml of blood (~1% of total leukocytes)



## Cell Separation Solutions

STEMCELL Technologies has developed a full range of Human Treg isolation kits aimed to meet the needs of Treg researchers.

### Our kits are:

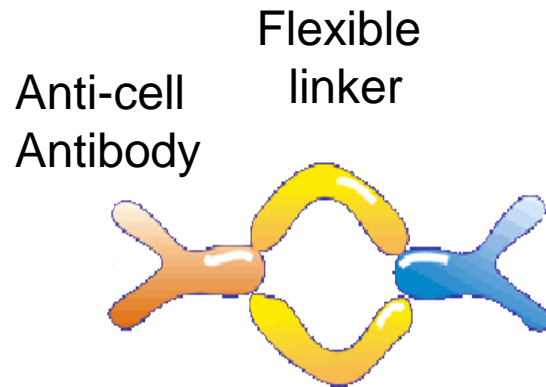
- Fast
- Easy
- Versatile



High Purity,  
Functional Tregs

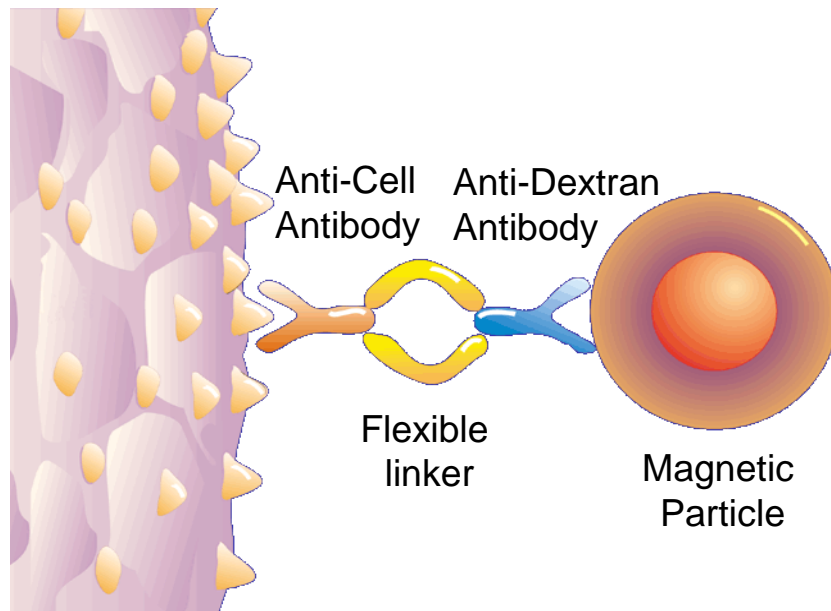
## Tetrameric Antibody Complex – (TAC)

TACs are formed by two mouse antibodies that held in opposition by a rat antibody that recognizes the Fc portion of a mouse IgG<sub>1</sub> antibody

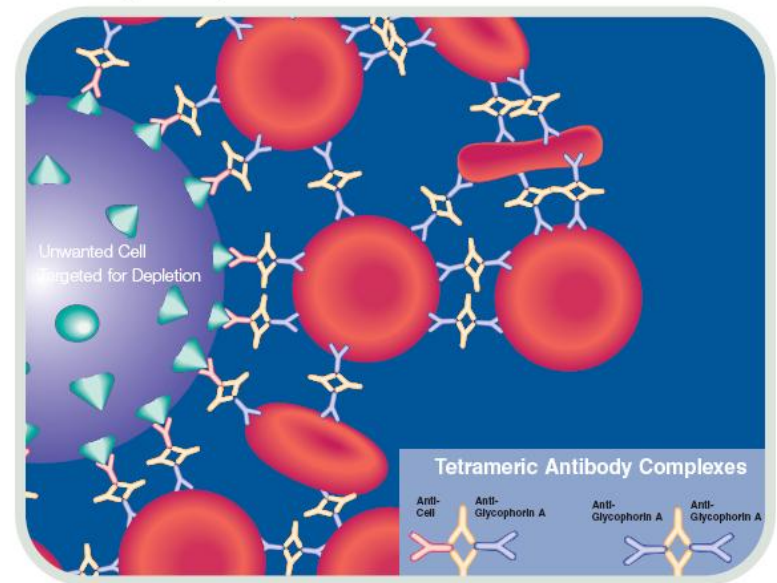




**+EasySep<sup>®</sup>**



**RosetteSep<sup>®</sup>**



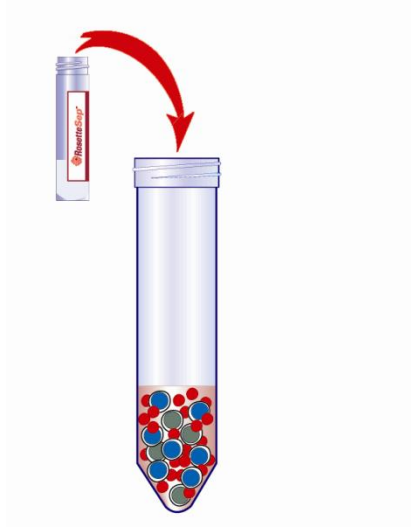


## Starting with Whole Blood or Buffy Coats



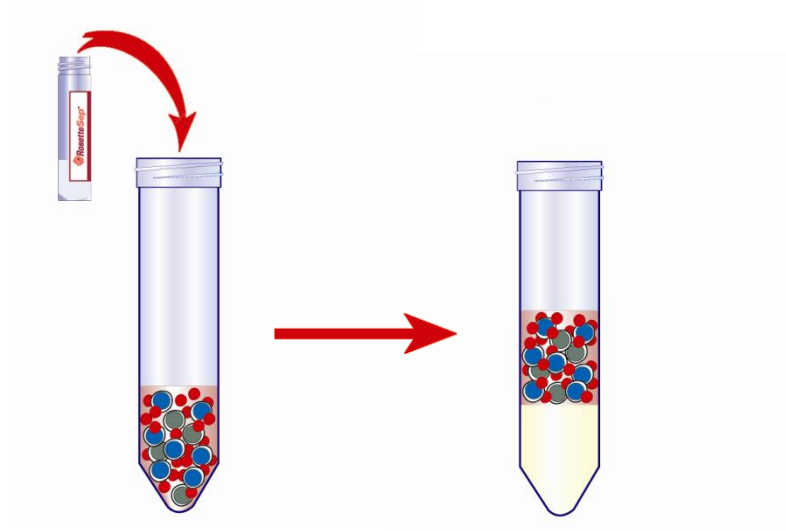
# **RosetteSep<sup>®</sup>** Immunodensity Centrifugation

1) Add RosetteSep<sup>®</sup> cocktail and incubate 20 minutes



# **RosetteSep<sup>®</sup>** Immunodensity Centrifugation

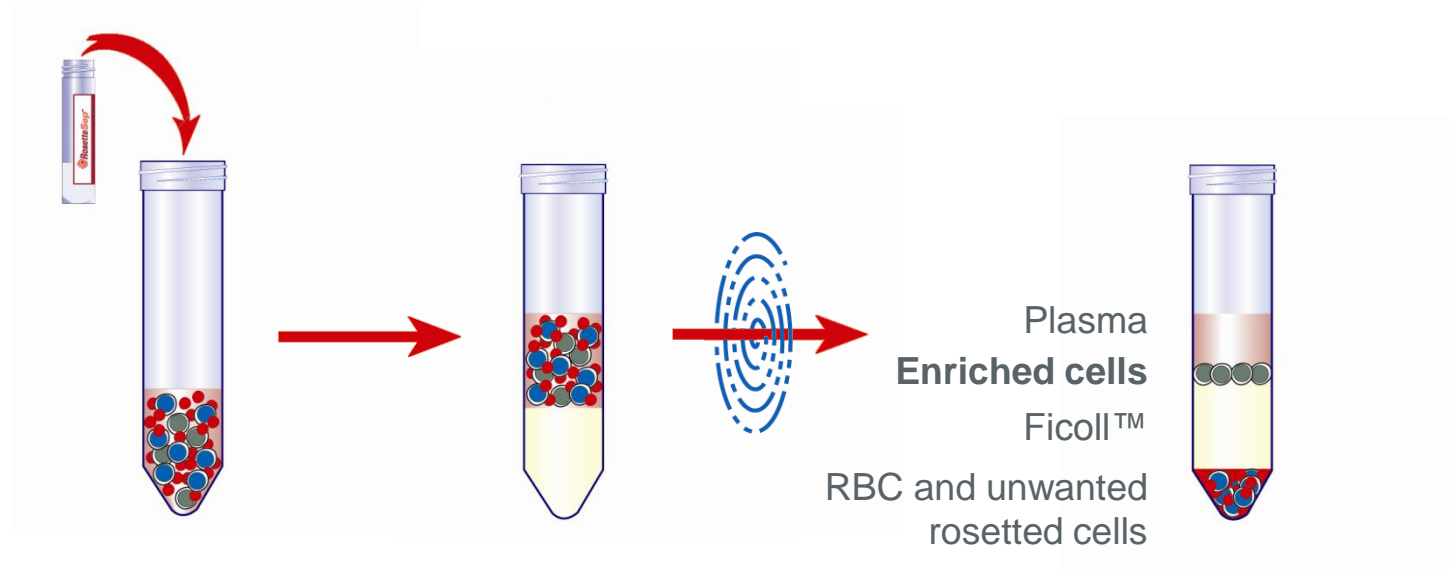
2) Dilute sample and layer over density medium (e.g. Ficoll™)





# Immunodensity Centrifugation

## 3) Spin

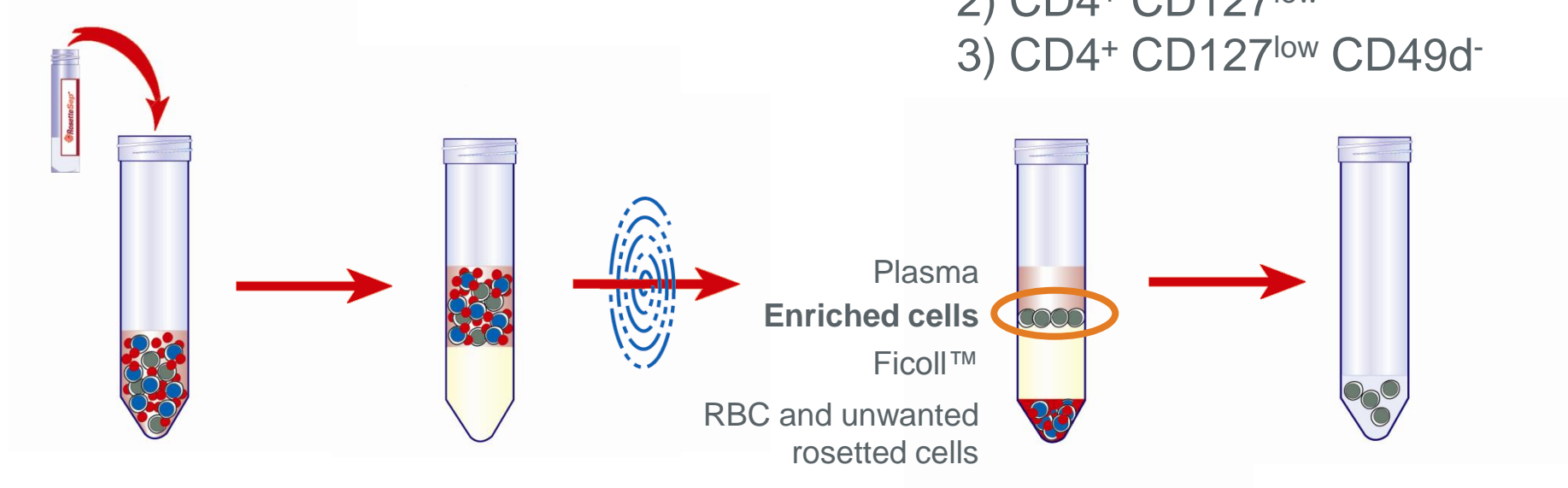


# **RosetteSep<sup>®</sup>** Immunodensity Centrifugation

## 4) Collect enriched cells

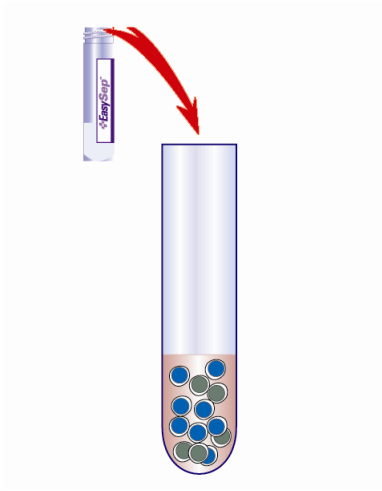
### Pre-enriched cells:

- 1) CD4<sup>+</sup>
- 2) CD4<sup>+</sup> CD127<sup>low</sup>
- 3) CD4<sup>+</sup> CD127<sup>low</sup> CD49d<sup>-</sup>



# +EasySep<sup>®</sup> Immunomagnetic Cell Separation

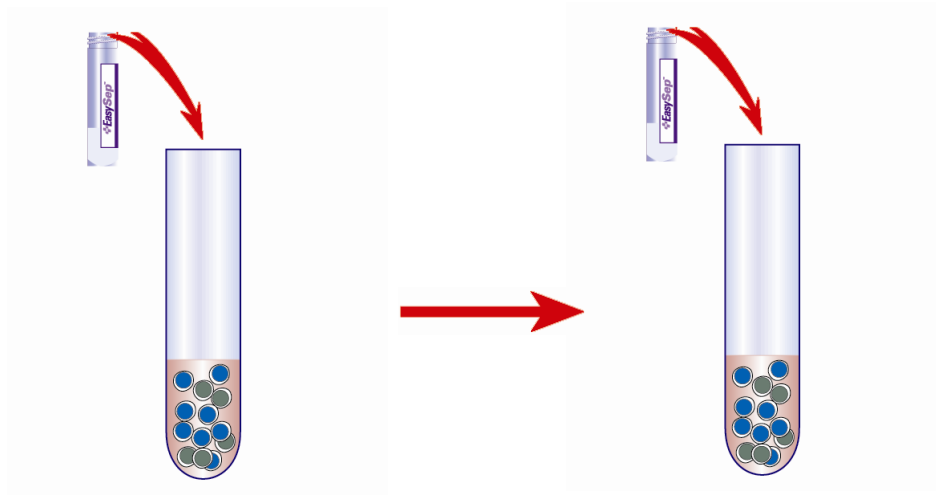
1) Add EasySep<sup>®</sup> selection cocktail and incubate 15 minutes





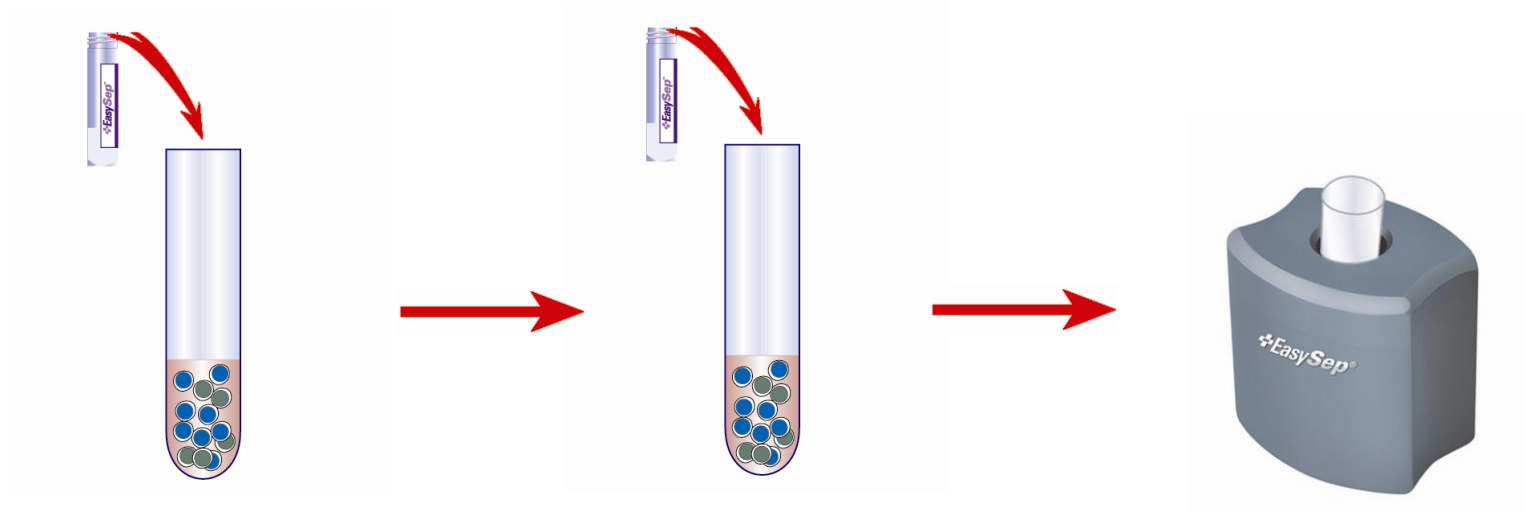
## **+EasySep<sup>®</sup>** Immunomagnetic Cell Separation

2) Add EasySep<sup>®</sup> magnetic particles and incubate 10 minutes



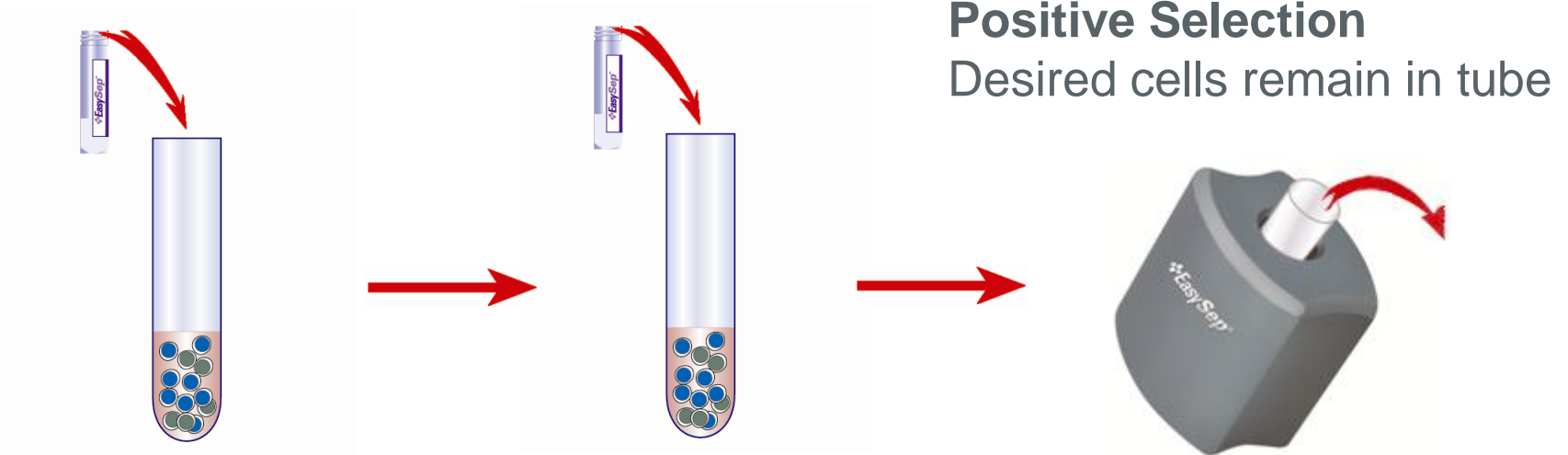
## **+EasySep<sup>®</sup>** Immunomagnetic Cell Separation

3) Place tube in magnet for 5 minutes



## **+EasySep<sup>®</sup>** Immunomagnetic Cell Separation

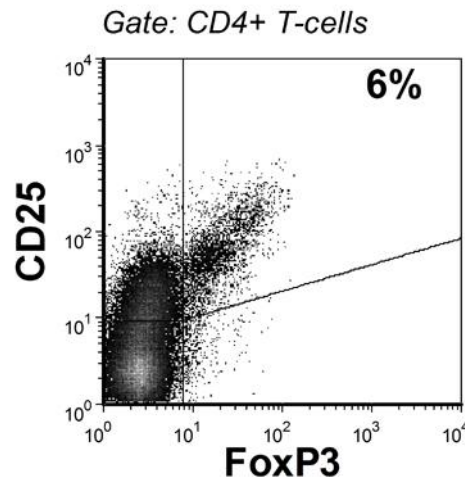
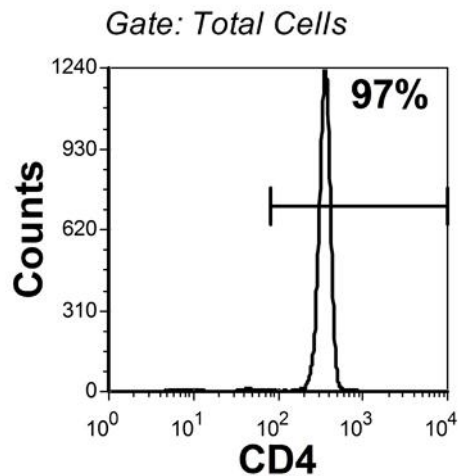
4) Pour off supernatant and wash cells three times



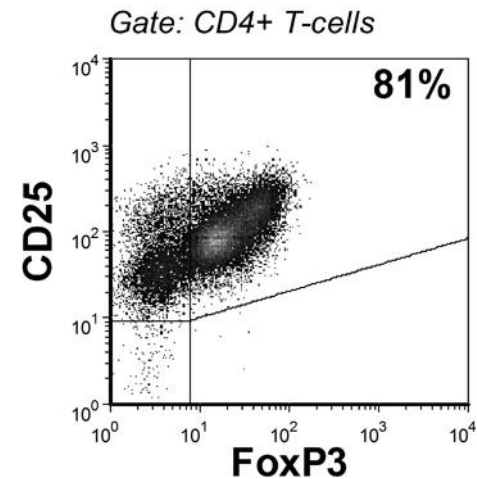
## “Classic” Tregs

Complete Kit for Human CD4<sup>+</sup> CD25<sup>+</sup> Regulatory T Cells (#15862)

Pre-enriched  **RosetteSep<sup>®</sup>**



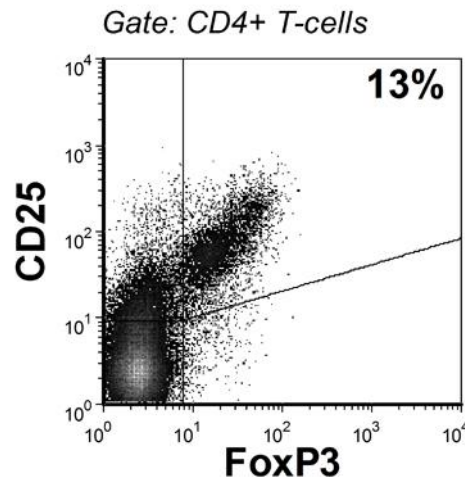
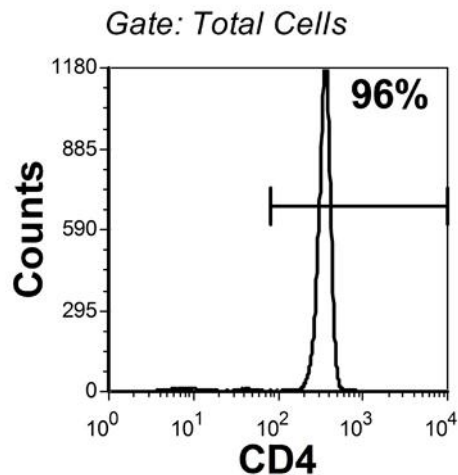
Enriched  **EasySep<sup>®</sup>**



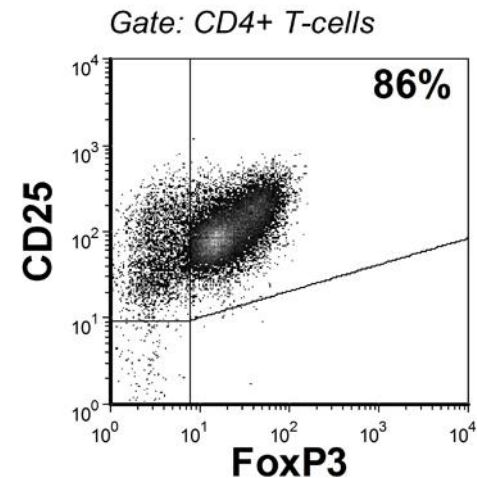
## Higher Purity Tregs

Complete Kit for Human  $CD4^+CD127^{low}CD25^+$  Regulatory T Cells  
(#15861)

Pre-enriched  **RosetteSep<sup>®</sup>**



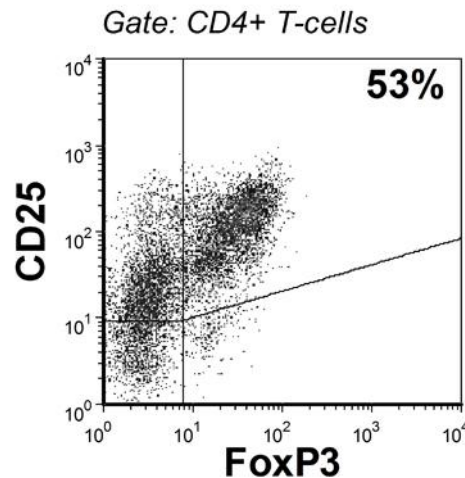
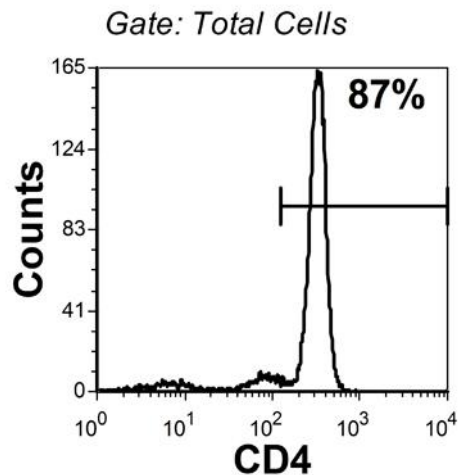
Enriched  **EasySep<sup>®</sup>**



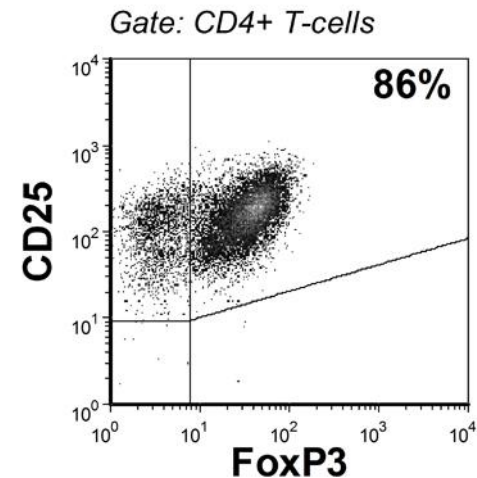
## Higher Purity Activated Tregs

Complete Kit for Human CD4<sup>+</sup> CD127<sup>low</sup> CD49d<sup>-</sup> CD25<sup>+</sup> Regulatory T Cells  
Catalog #15864

Pre-enriched  **RosetteSep<sup>®</sup>**

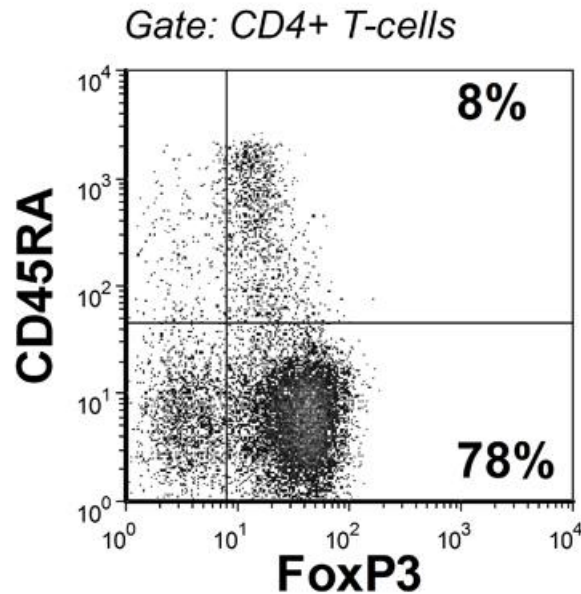


Enriched  **EasySep<sup>®</sup>**

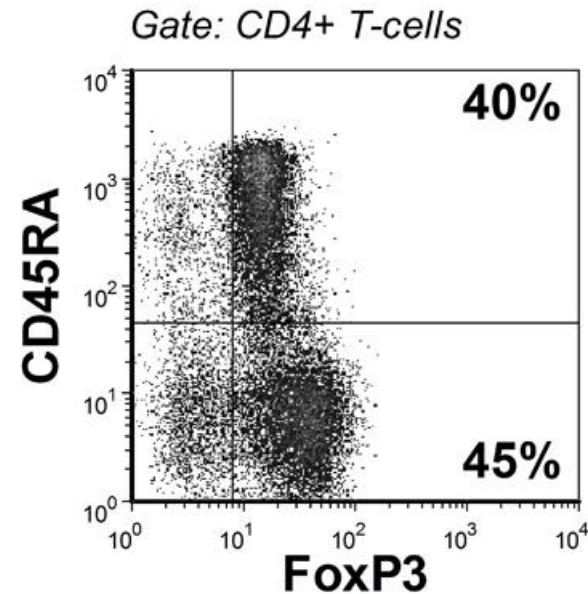




## CD49d<sup>-</sup> Tregs have an Activated Phenotype

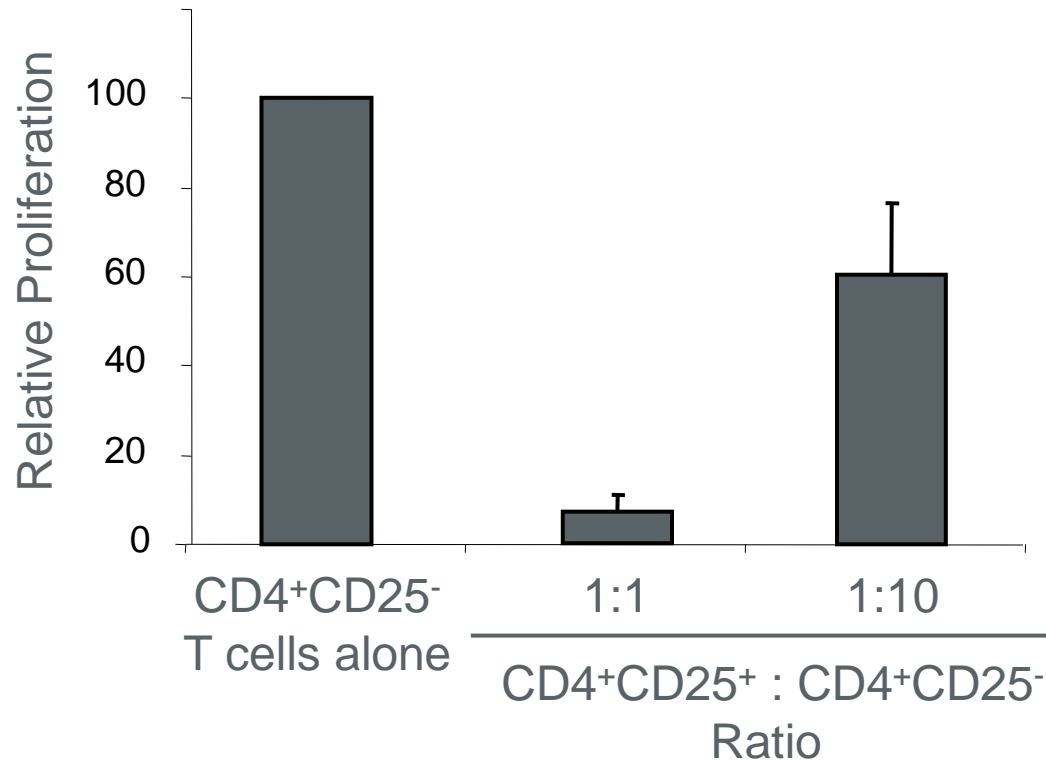


CD4<sup>+</sup> CD127<sup>low</sup> CD49d<sup>-</sup> CD25<sup>+</sup>

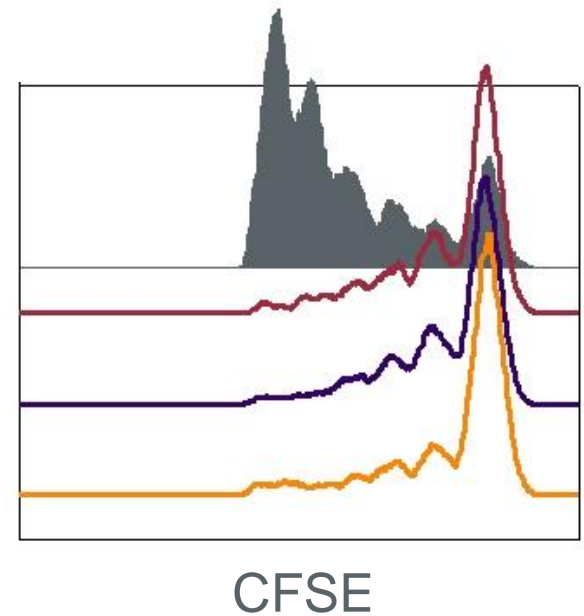
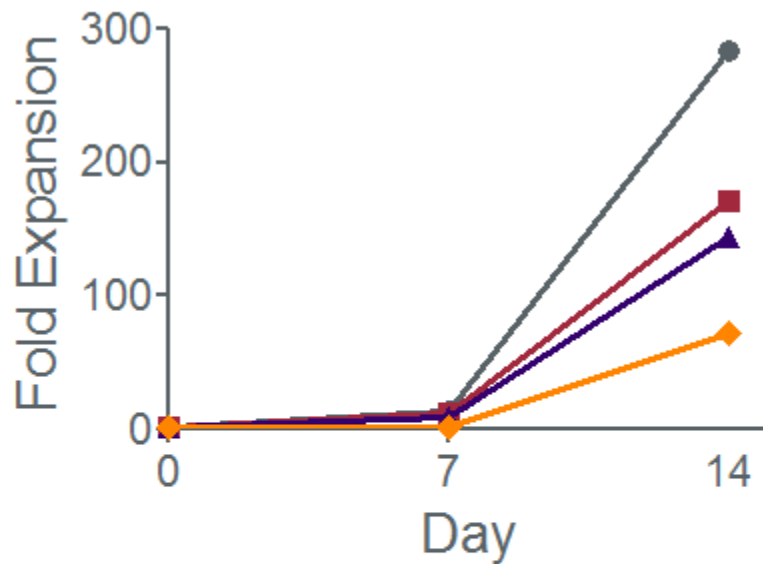


CD4<sup>+</sup> CD127<sup>low</sup> CD25<sup>+</sup>

## Freshly Isolated CD4<sup>+</sup>CD25<sup>+</sup> T cells Suppress Proliferation of CD4<sup>+</sup>CD25<sup>-</sup> Responder Cells



# Expanded Tregs Suppress Proliferation of CD4<sup>+</sup>CD25<sup>-</sup> Responder Cells



No Rapamycin

● CD4<sup>+</sup>CD25<sup>-</sup>

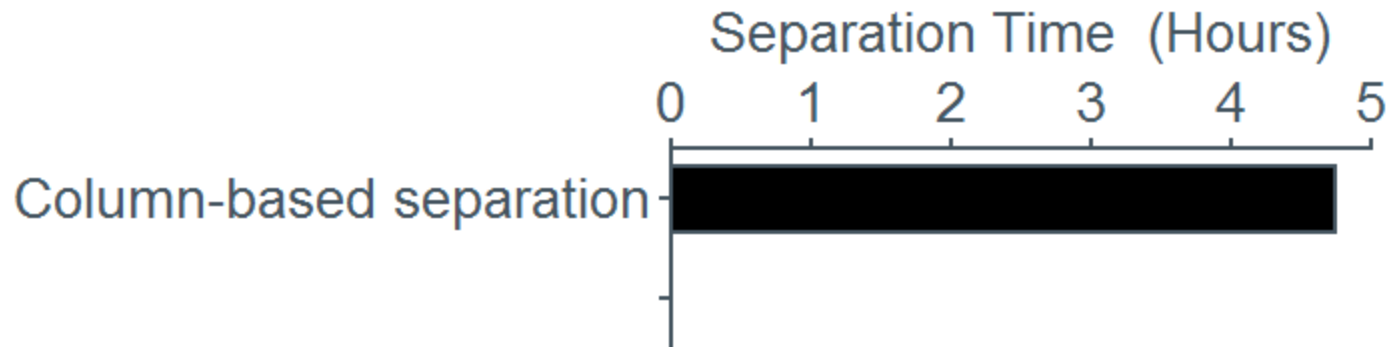
100nM Rapamycin

■ CD4<sup>+</sup>CD25<sup>+</sup>

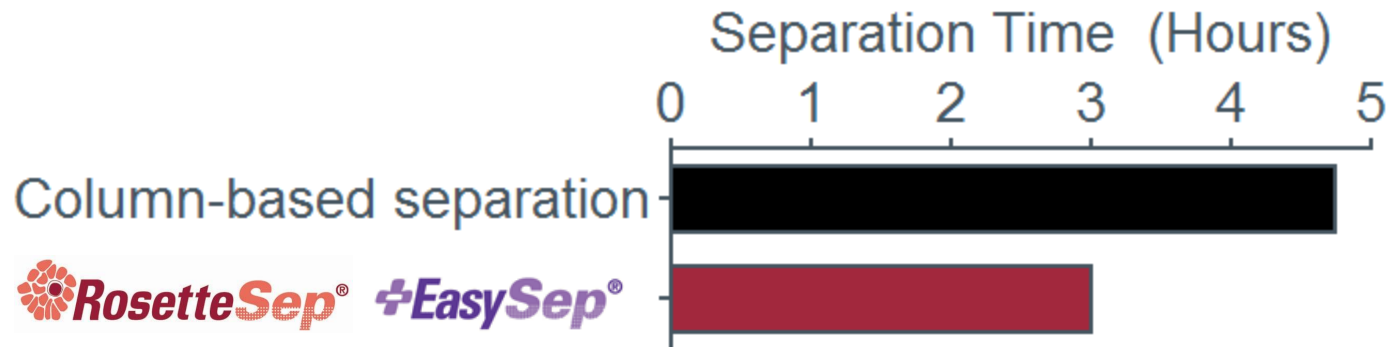
▲ CD4<sup>+</sup>CD127<sup>low</sup>CD25<sup>+</sup>

◆ CD4<sup>+</sup>CD127<sup>low</sup>CD49d<sup>-</sup>CD25<sup>+</sup>

## Significant Time Savings

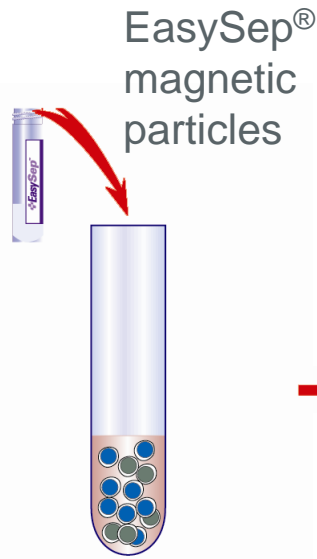
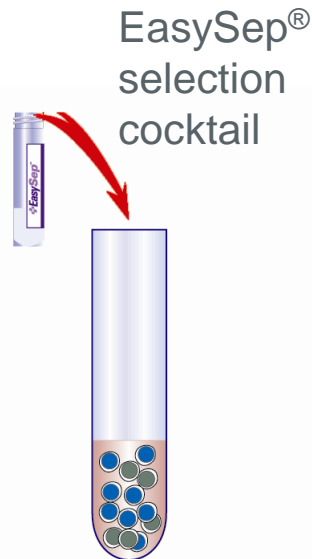


## Significant Time Savings



# EasySep<sup>®</sup> CD4<sup>+</sup>CD25<sup>+</sup> Regulatory T Cell Isolation Kit

**45 minutes** for EasySep<sup>®</sup> CD4<sup>+</sup> T cell pre-enrichment



## Negative Selection

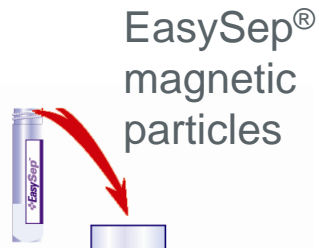
Desired cells are poured off  
into a new tube





# EasySep<sup>®</sup> CD4<sup>+</sup>CD25<sup>+</sup> Regulatory T Cell Isolation Kit

45 minutes for EasySep<sup>®</sup> CD25 positive selection

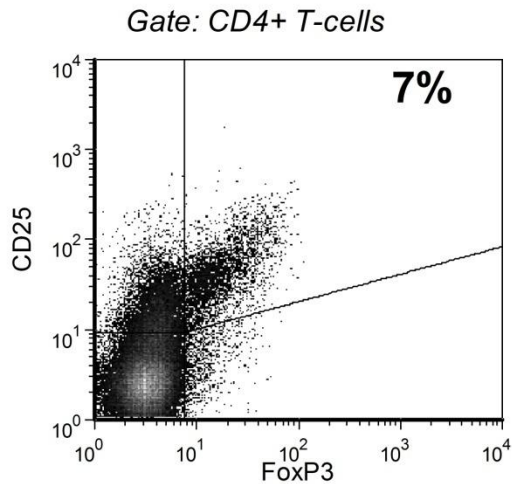


**Positive Selection**  
Desired cells remain in tube

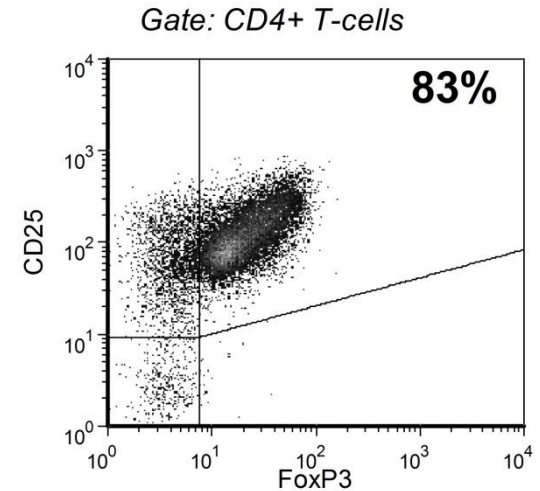
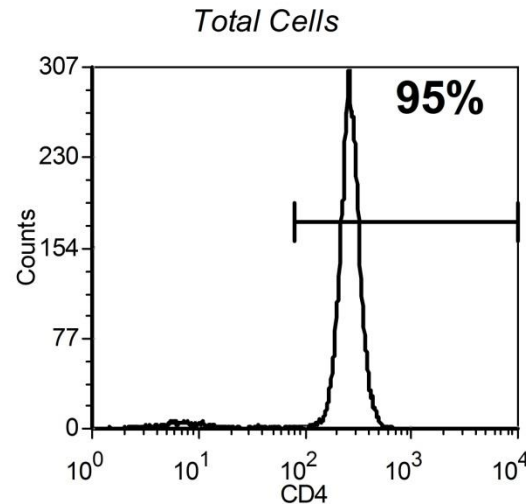


# Two Step Isolation of CD4<sup>+</sup>CD25<sup>high</sup> T Cells from PBMC with EasySep<sup>®</sup>

Start



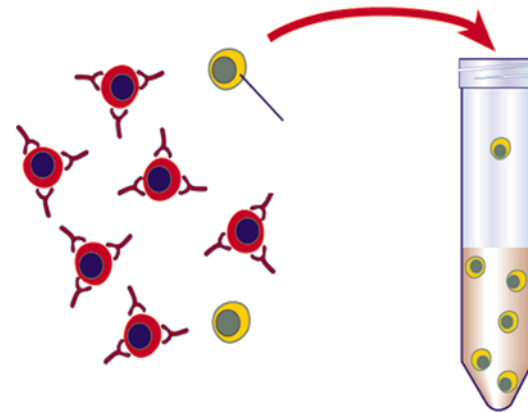
Enriched



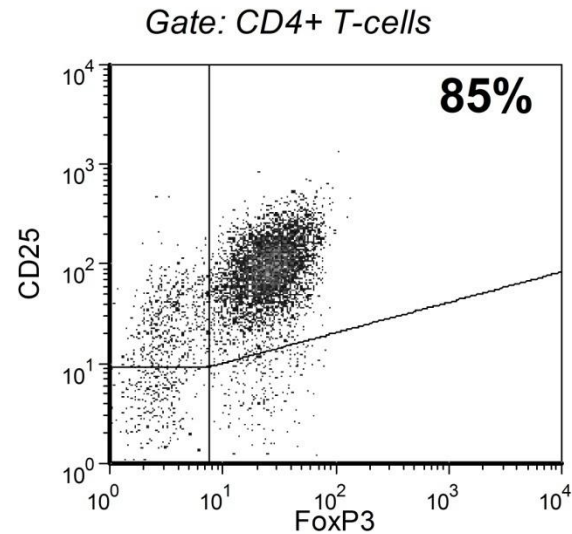
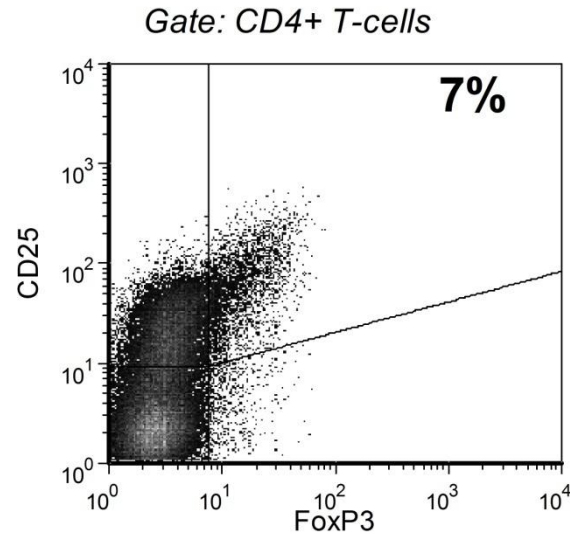
# Isolation of Untouched Tregs

## FAST, EASY and GENTLE

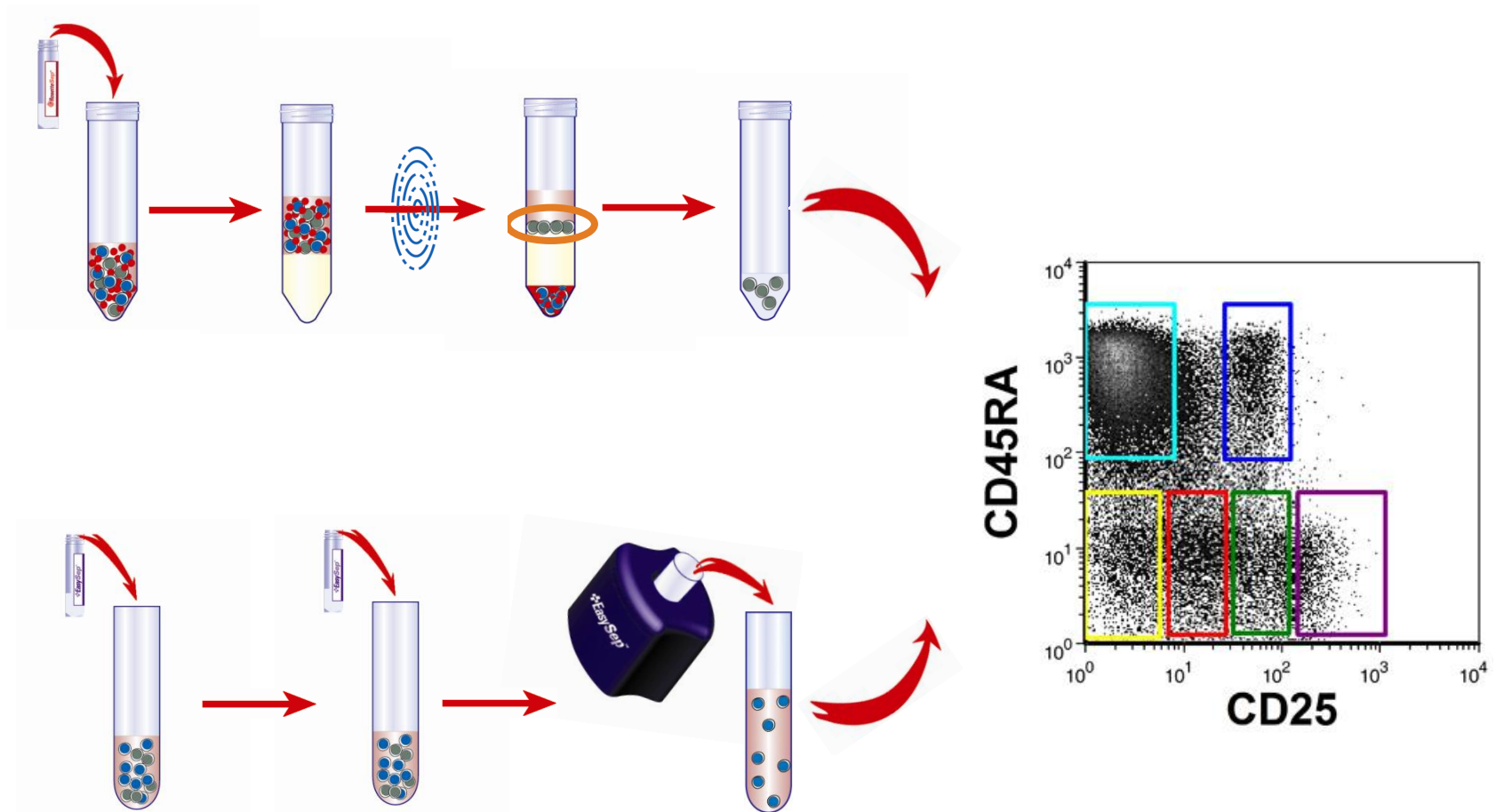
Get highly functional, untouched Tregs in a single step from PBMCs in one hour.



# EasySep<sup>®</sup> CD4<sup>+</sup>CD127<sup>low</sup>CD49d<sup>-</sup> Regulatory T Cell Enrichment Kit

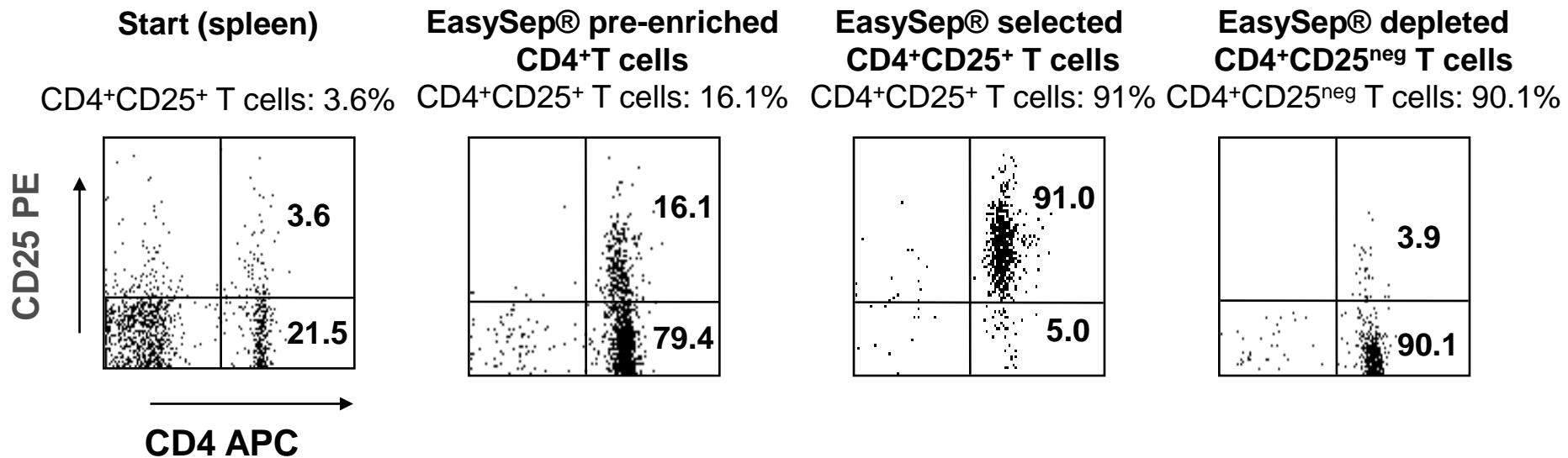


# Pre-Enrichment of Tregs for FACSorting



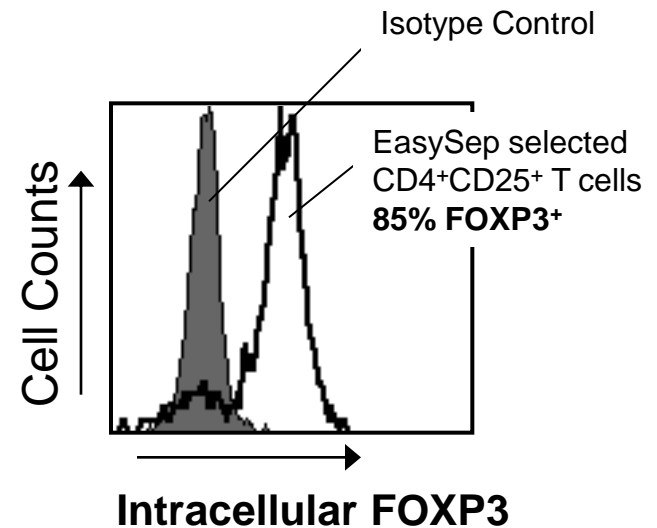
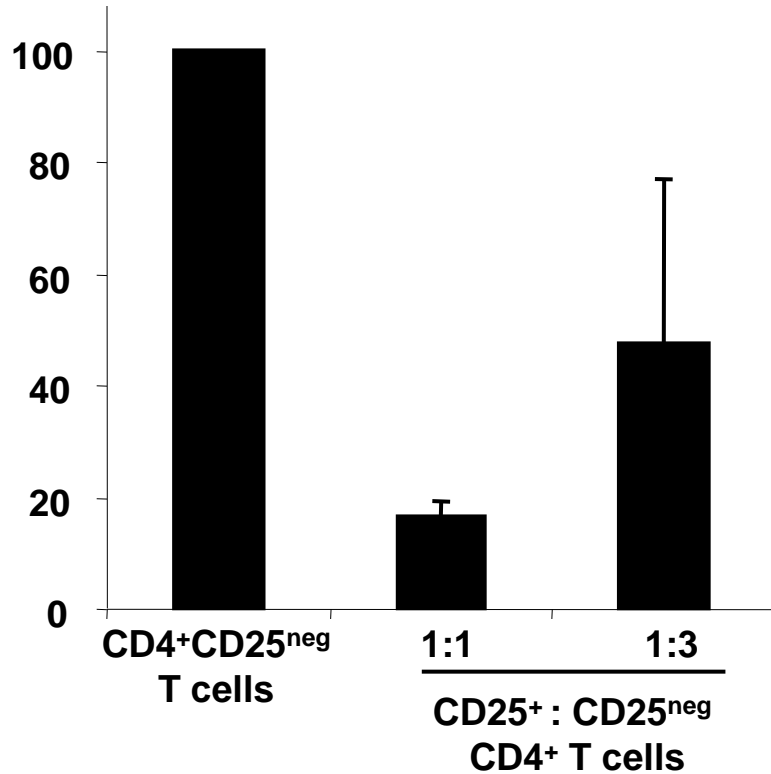


# Isolation of Mouse CD4<sup>+</sup>CD25<sup>+</sup> Treg Cells





# Mouse CD4<sup>+</sup>CD25<sup>+</sup> Cells Suppress T Cell Proliferation



## Customize Your Cell Separation

- Can't find the cell isolation kit you need?
- STEMCELL offers custom kits, reagents, protocols and technical expertise to create tailor-made products for your specific research needs
- Ideal for:
  - Rare cell types
  - Non-standard species
  - Special applications

## Cell Separation Solutions from STEMCELL

Fast

Easy-to-use

Gentle

Versatile

STEMCELL will work with you to get the Cell Separation Solution that you need for your research



Any questions?

[www.stemcell.com](http://www.stemcell.com)  
[info@stemcell.com](mailto:info@stemcell.com)