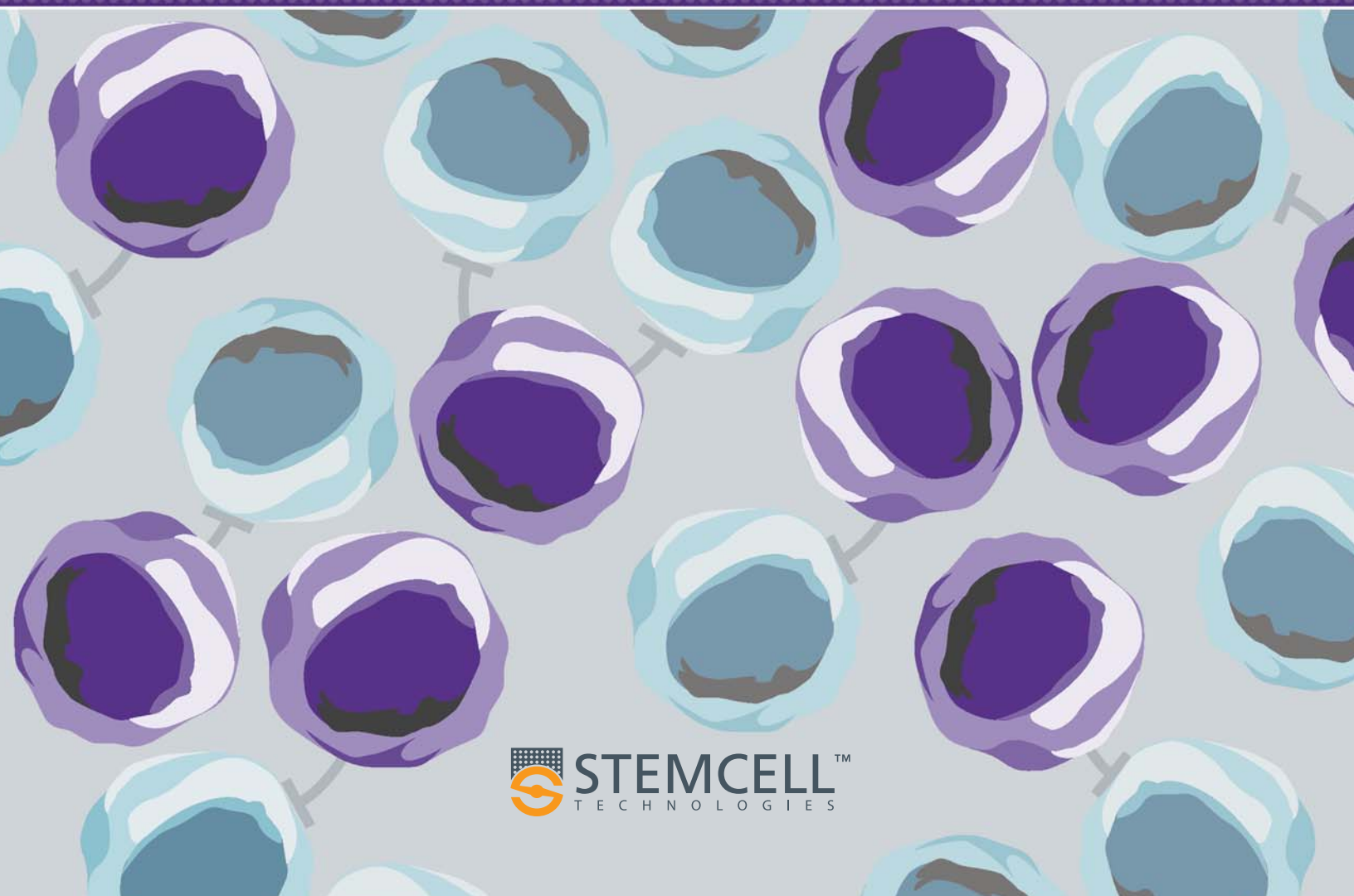
Cell Isolation Products For

Regulatory T Cells



STEMCELL™
TECHNOLOGIES

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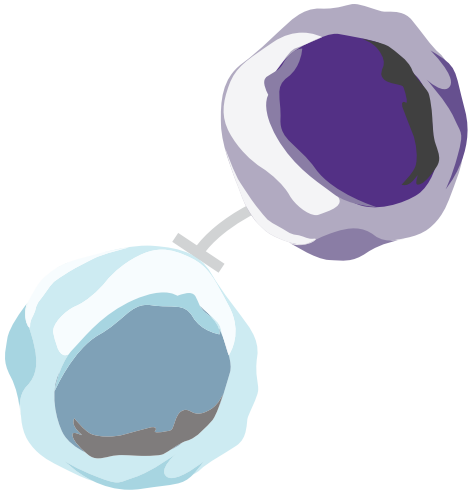
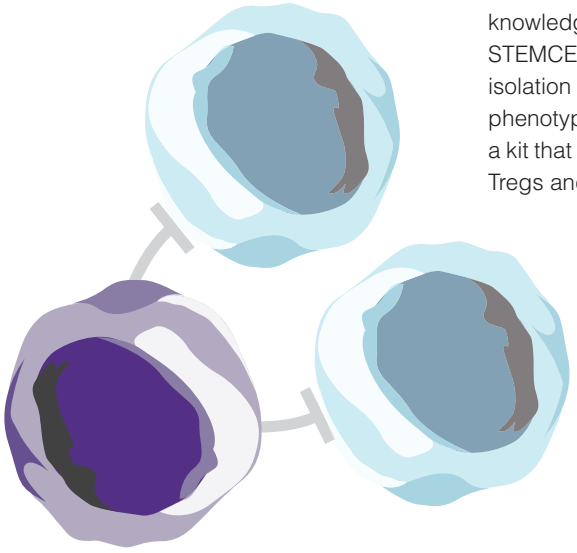
Your First Step Towards

Cutting-Edge Treg Research



Regulatory T cells (Tregs) are a specialized subset of T cells that play an essential role in the prevention of autoimmunity and the maintenance of immune system homeostasis and tolerance to self antigens. Harnessing the suppressive activity of Tregs for therapeutic purposes is a major area of interest as they hold great potential for the treatment of autoimmune diseases, prevention of transplant rejection and cancer immunotherapy.



The first step towards cutting-edge Treg research is the isolation of highly purified, functional Tregs. With unique cell isolation platforms, a team of dedicated and knowledgeable scientists, and a collaborative network of academic researchers, STEMCELL Technologies has developed a full range of products for the rapid and efficient isolation of highly functional Tregs. Choose the best Treg kit based on your desired phenotype, your downstream application and your starting sample, or let us design a kit that is tailor-made for your research. Start your Treg research right with high purity Tregs and STEMCELL Technologies.

Regulatory T Cells

Regulatory T cells (Tregs) have the ability to suppress T cell responses and play a critical role in peripheral tolerance and immune regulation.[1] In humans, natural Tregs are phenotypically $CD4^+CD25^{high}$, and express high levels of the intracellular transcription factor FoxP3.[2,3] Unlike their mouse counterparts, human Tregs appear to display a heterogeneous cell surface phenotype and regulatory capacity, leading to the identification and classification of various Treg subtypes within the $CD4^+CD25^{high}FoxP3^+$ T cell population.[4] Human peripheral blood Tregs are rare, comprising only 2 – 3% of the $CD4^+$ T cell population.[2] For these reasons, the identification and isolation of human Tregs can be challenging and time-consuming.

To overcome these technical difficulties, researchers have exploited the expression of other surface markers, either alone or in combination, in order to enrich for distinct Treg populations. The expression of CD127 (IL-7 receptor alpha chain) inversely correlates with FoxP3 expression,[6,7] while CD49d, the α-chain of the integrin VLA-4 ($\alpha_4\beta_1$) is expressed on a majority of pro-inflammatory effector cells.[8] Removal of $CD127^{high}$ cells within the $CD4^+CD25^{high}$ population enriches for a subset of Tregs with high levels of FoxP3 expression and high expansion potential,[7] whereas the use of CD127 and CD49d antibodies in combination provides a negative selection (e.g. depletion) approach to isolate purified untouched Tregs with suppressive activity.[7,8]

More recently, new subpopulations of Tregs have been identified based on the expression of CD45RA.[4,9,10] The heterogeneous expression of CD45RA allows for the identification and isolation of Treg subtypes that are functionally distinct and have different suppression and expansion capabilities. These subtypes include resting Tregs, activated Tregs, and cytokine-secreting Tregs.

In order to support and facilitate research in this exciting field, STEMCELL Technologies has developed cutting-edge products for the isolation and assessment of Tregs. Our Treg isolation kits provide the fastest and easiest way to isolate various subtypes of Tregs directly from human whole blood, buffy coat, PBMC or from mouse spleen with high purity and recovery. Purified cells are highly functional and ready to use in any downstream Treg assay.

The STEMCELL
Advantage

FAST

- Save hours on a complete separation
- Isolate cells directly from whole blood, buffy coat or leukapheresis samples

COST-EFFECTIVE

- No expensive columns
- Save time on costly flow cytometry sorting
- Kits are stable for up to two years

VERSATILE

- Isolate different subtypes of Tregs
- Choose the best kit for your downstream application or starting sample
- Customize kits to get the Tregs you want

Did you know?

EasySep™ cell isolation can be fully automated with RoboSep™, which can isolate from four samples simultaneously.

Treg Isolation Procedure

Isolate Tregs directly from whole blood or buffy coat using our **Complete Kits for Regulatory T Cells**. Tregs are first pre-enriched during a Ficoll-Paque™ PLUS spin using RosetteSep™ (Step 1). The CD25^{high} cells are then positively selected using EasySep™ immunomagnetic separation system (Step 2).

For isolation of CD4^{+}CD25^{high} Tregs from PBMCs, Tregs are pre-enriched with EasySep™ instead of RosetteSep™, followed by the EasySep™ CD25^{high} positive selection procedure (Step 2).

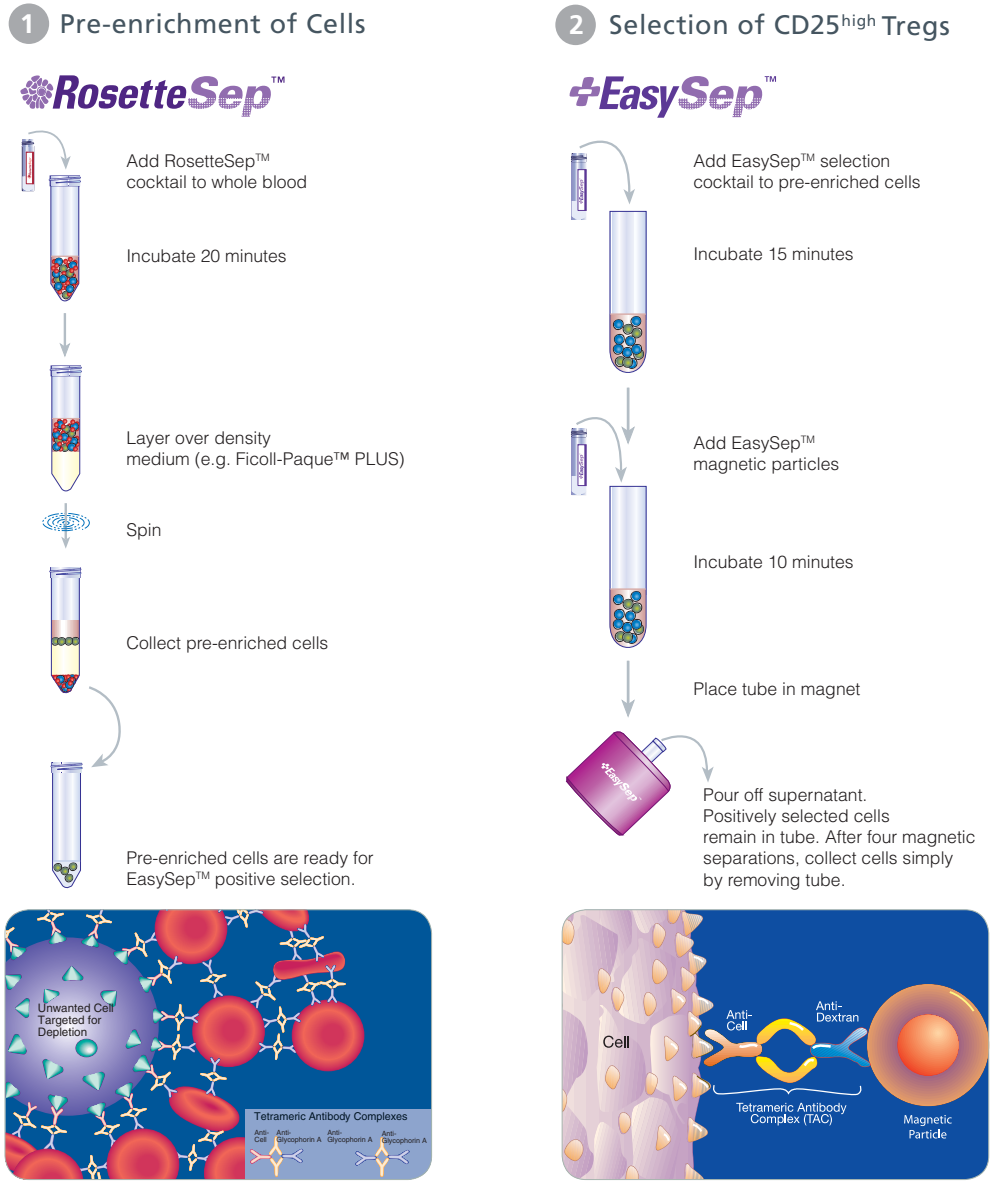


FIGURE 1. RosetteSep™ Tetrameric Antibody Complexes (TAC)

FIGURE 2. EasySep™ Tetrameric Antibody Complexes (TAC)

FIGURE 1. Unwanted cells are crosslinked to red blood cells (RBC) using bispecific Tetrameric Antibody Complexes (TACs). These labeled cells pellet with the RBC when centrifuged over density medium, leaving the enriched CD4^{+} T cells at the plasma:density medium interface.

FIGURE 2. TACs selectively bind to desired cells (positive selection) or unwanted cells (negative selection) and crosslink them to magnetic particles. The labeled cells are then separated from unlabeled cells using the EasySep™ magnet.

Significant Time Savings

Protocol Comparison Between Treg Isolation Kits by STEMCELL Technologies and a Competitor

	STEMCELL TECHNOLOGIES	COMPETITOR
Time	**Complete Kits for Human Regulatory T Cell Isolation**	**Column-based Human Regulatory T Cell Isolation Kits**
0:00	RosetteSep™ $CD4^+$ or $CD4^+$ subset cocktail incubation	
0:15		Ficoll-Paque™ PLUS spin
0:30	Ficoll-Paque™ PLUS spin	
0:45		Wash cells x 2
1:00	Wash cells x 2	
1:15		$CD4^+$ biotin cocktail incubation
1:30	Resuspend in buffer	Microbead incubation
1:45	CD25 cocktail incubation	Centrifuge
2:00	Magnetic particle incubation	Load and run column
2:15	Magnetic separation	Wash column x 2
2:30		
2:45	Wash cells	Wash cells
3:00	**Tregs ready for use**	CD25 microbead incubation
3:15		Wash cells
3:30		Load and run column
3:45		Wash column x 3
4:00		Load and run column
4:15		Wash column x 3
4:30		Wash cells
4:45		**Tregs ready for use**

3 hr
No columns
4 centrifugation steps

4 hr 45 min
3 columns
9 centrifugation steps

Classic Tregs

Initial phenotypic analysis of human Tregs showed that in vitro regulatory activity is restricted to $CD4^+$ T cells that express high levels of CD25.[2,3] Isolation of $CD4^+CD25^{high}$ T cells allows researchers to isolate and study the broadest phenotypic range of Tregs.

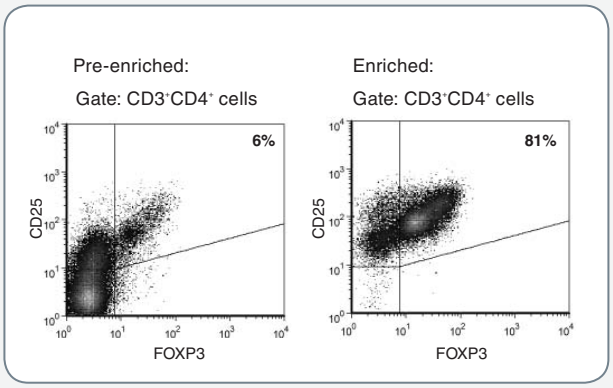
STEMCELL Technologies' kits provide the **fastest** isolation of highly purified $CD4^+CD25^{high}$ T cells. These cells can be used either directly in downstream assays, or as a pre-enriched population for reducing flow sorting time.

Complete Kit for Human $CD4^+CD25^{high}$ T Cells (CATALOG #15862)

Starting with whole blood, isolate $CD4^+CD25^{high}$ T cells quickly and easily in two simple steps.

HOW IT WORKS:

$CD4^+$ T cells are enriched by negative selection using RosetteSep™ followed by positive selection of $CD25^{high}$ cells using EasySep™ or RoboSep™ (see page 6).



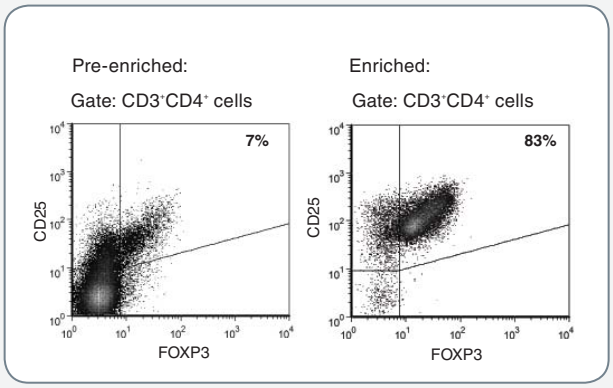
Purity and phenotype of $CD3^+CD4^+$ cells following pre-enrichment with RosetteSep™ ("Pre-enriched") and positive selection with EasySep™ ("Enriched").

EasySep™ Human $CD4^+CD25^{high}$ T Cell Isolation Kit (CATALOG #18062)

Starting with PBMC, isolate $CD4^+CD25^{high}$ T cells without columns or washes.

HOW IT WORKS:

$CD4^+$ T cells are enriched by negative selection using EasySep™ or RoboSep™ followed by positive selection of $CD25^{high}$ cells using EasySep™ or RoboSep™.



Purity and phenotype of $CD3^+CD4^+$ cells following pre-enrichment with EasySep™ ("Pre-enriched") and positive selection with EasySep™ ("Enriched").

Highest Purity Tregs

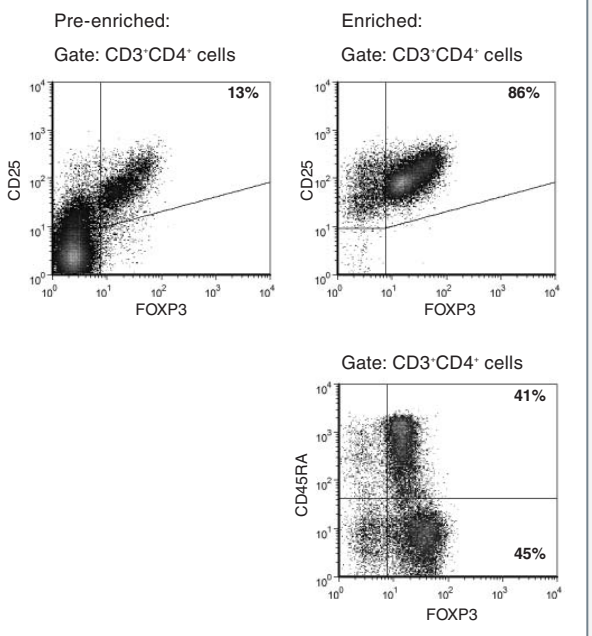
CD127 and CD49d are useful cell surface markers for removing effector cells from $CD4^+CD25^+$ T cell populations allowing for further enrichment of highly suppressive Tregs.[6,7,8] STEMCELL has developed kits using these markers to give you the highest purity of $FoxP3^+$ cells for downstream analysis without the need for additional flow sorting.

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

Get high purity and recovery of $FoxP3^+$ Tregs directly from whole blood.

HOW IT WORKS:

$CD4^+CD127^{low}$ T cells are enriched by negative selection using RosetteSep™ followed by positive selection of $CD25^{high}$ cells using EasySep™ or RoboSep™ (see page 6).



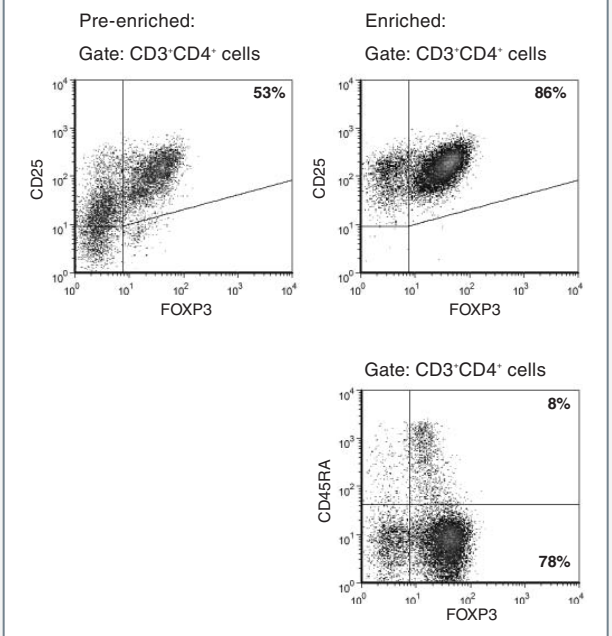
Purity and phenotype of $CD3^+CD4^+$ cells following pre-enrichment with RosetteSep™ ("Pre-enriched") and positive selection with EasySep™ ("Enriched").

Complete Kit for Human $CD4^+CD127^{low}CD49d^-CD25^{high}$ Regulatory T Cells (CATALOG #15864)

Get the highest purity of $FoxP3^+$ activated/memory Tregs directly from whole blood.

HOW IT WORKS:

$CD4^+CD127^{low}CD49d^-$ T cells are enriched by negative selection using RosetteSep™ followed by positive selection of $CD25^{high}$ cells using EasySep™ or RoboSep™ (see page 6).



Purity and phenotype of $CD3^+CD4^+$ cells following pre-enrichment with RosetteSep™ ("Pre-enriched") and positive selection with EasySep™ ("Enriched").

Untouched Tregs

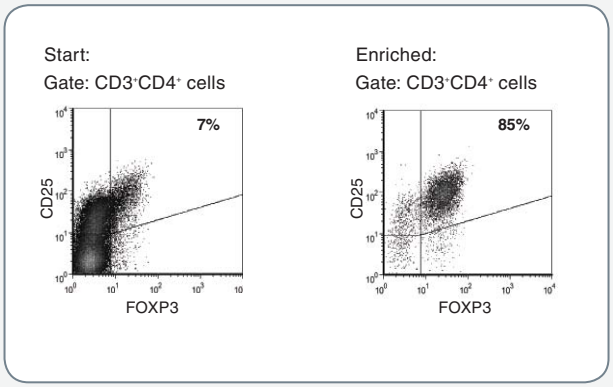
For researchers who prefer a gentle method of isolating untouched Tregs, STEMCELL has developed negative selection kits that enrich Tregs without the need for positive selection. Our EasySep™ isolation kits give highly functional, untouched Tregs in a single step from PBMCs in one hour.

EasySep™ Human CD4$^+$CD127^{low}CD49d$^-$ Regulatory T Cells (CATALOG #19232)

Isolate untouched FoxP3$^+$ T cells in a single step from PBMC.

HOW IT WORKS:

CD4$^+$CD127^{low}CD49d$^-$ T cells are enriched by EasySep™ negative selection, which depletes unwanted cells and leaves desired cells untouched.



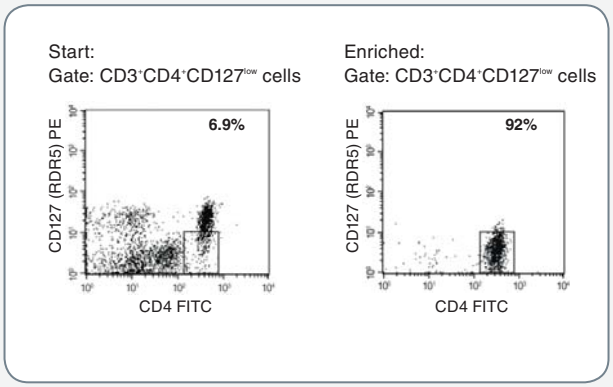
Purity and phenotype of CD3$^+$CD4$^+$ cells before ("Start") and after ("Enriched") EasySep™.

EasySep™ Human CD4$^+$CD127^{low} T Cells (CATALOG #19231)

Isolate untouched CD4$^+$CD127^{low} T cells in a single step from PBMC and save time on flow sorting.

HOW IT WORKS:

CD4$^+$CD127^{low} T cells are enriched by EasySep™ negative selection, which depletes unwanted cells and leaves desired cells untouched.



Purity and phenotype of CD3$^+$CD4$^+$ cells before ("Start") and after ("Enriched") EasySep™.

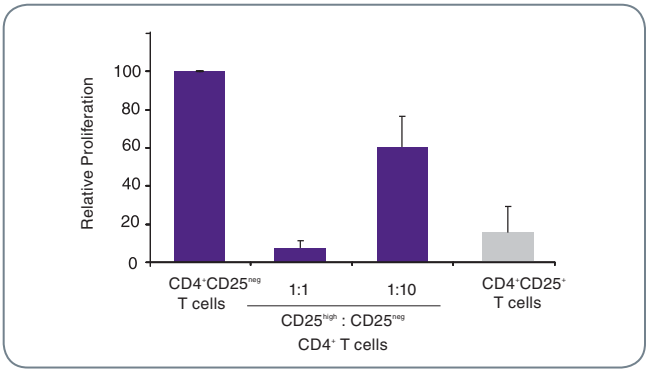
Isolate Tregs for

Functional Analysis

The suppression activity of isolated $CD4^+CD25^{high}$ T cells was assessed by measuring their impact on $CD4^+CD25^{neg}$ proliferative responses to stimulation with anti-CD3/CD28 coated beads in the absence of IL-2 (Figure 3). Mixing purified $CD4^+CD25^{high}$ T cells with $CD4^+CD25^{neg}$ T cells at a ratio of 1:1 almost completely eliminates detectable $CD4^+CD25^{neg}$ T cell proliferation responses, and suppression of proliferation continues to be detected when cells are mixed in a ratio of 1:10.

Next, Tregs isolated with RosetteSep™ and EasySep™ were first expanded ex vivo for 14 days in the presence of rapamycin, and then co-cultured with autologous $CD4^+CD25^{depleted}$ responder T cells labelled with CFSE at a 1 Treg : 2 responders ratio (Figure 4). Whereas, $CD4^+CD25^-$ T cells expanded in the absence of rapamycin have no suppressive capacity, all of the expanded Tregs effectively suppressed proliferation of the autologous $CD4^+CD25^{depleted}$ responder cells.

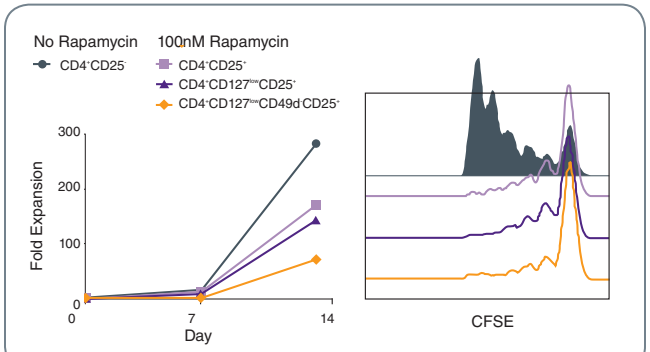
FIGURE 3. Isolated $CD4^+CD25^{high}$ T cells are anergic and suppress proliferation of $CD4^+CD25^{neg}$ T cells



Purified $CD4^+CD25^{high}$ T cell fractions were assessed for anergy by measuring their proliferation response to anti-CD3/CD28 coated beads (in grey). The suppression activity of purified $CD4^+CD25^+$ T cells was assessed by measuring their ability to reduce the proliferative response of $CD4^+CD25^{neg}$ T cells to CD3/CD28 beads (in black). T cell proliferation was quantified by measuring dilution of the fluorescent dye CFSE with flow cytometry. Results are expressed as proliferation detected relative to control $CD4^+CD25^{neg}$ cell populations stimulated with CD3/CD28 beads for 7 days in RPMI medium containing 5% human AB serum. The bars are the means of 5 experiments with error bars indicating standard deviations.

Acknowledgements:
We would like to thank Dr. Rajendra Pahwa (Diabetes Research Institute, University of Miami Miller School of Medicine) for samples and technical assistance during the development of some RoboSep™ protocols.

FIGURE 4. Expanded Tregs Suppress Proliferation of $CD4^+CD25^-$ Responder Cells



Left: Freshly isolated Tregs were stimulated with anti-CD3/CD28 coated beads in the presence of exogenous IL-2 and 100 nm rapamycin. On day 7 the cells were restimulated and the cultures continued until day 14. The $CD4^+CD25^-$-depleted cells in grey, were cultured in the absence of rapamycin for the duration of culture.
Right: Following the 14 days of culture, the expanded Tregs were co-cultured with autologous $CD4^+CD25$-depleted responder T cells labelled with CFSE at a 1 Treg : 2 responders ratio.

STEMCELL's $CD4^+CD25^{high}$ Regulatory T Cell Isolation Kit and related products have been isolated for:

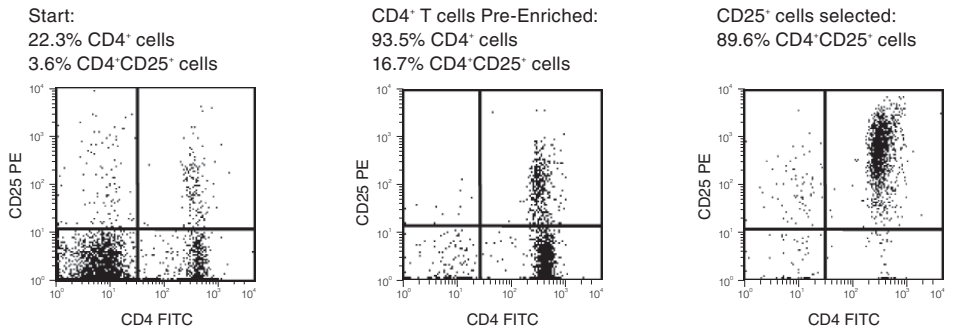
- Activation and expansion[1,2,3,4]
- Suppression of allogeneic mixed lymphocyte response (MLR)[1,5]
- Co-culture with dendritic cells[2,5,6]
- Adoptive transfer[4,7]
- RNA extraction[3,8]
- Gene expression profile[8]
- Real-time PCR analysis[8,5]
- Chromatin immunoprecipitation–DNA microarray (ChIP-chip)[8]

Isolation of

Mouse Tregs

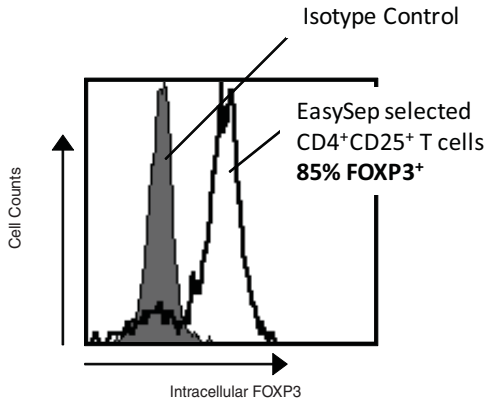
Mouse regulatory T cells can be isolated from single cell suspensions of spleen or other tissues using EasySep™ Mouse CD4+CD25+ Regulatory T Cell Isolation Kit (Catalog #19782). Mouse CD4+ T cells are enriched using EasySep™ Mouse CD4+ T Cell Pre-Enrichment Kit (Catalog #19772), which depletes cells expressing CD8, CD11b, CD19, CD24, CD45R, and CD49b. CD25+ cells are labeled using EasySep™ anti-CD25 PE Labeling Reagent, PE Selection Cocktail, and EasySep™ Magnetic Particles, and separated from unlabeled cells using an EasySep™ magnet.

FIGURE 5. Typical FACS Analysis of EasySep™ Mouse CD4+CD25+ Regulatory T Cell Isolation Kit



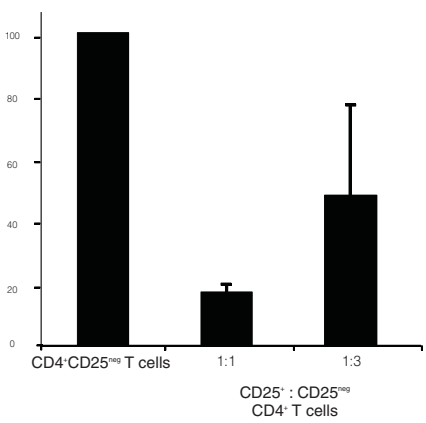
Starting with mouse splenocytes, the CD4+CD25+ cell content of the selected cells typically ranges from 85 - 97%.

FIGURE 6. EasySep™ isolated CD4+CD25+ T cell fractions are highly enriched for FoxP3-expressing cells.



Intracellular FoxP3 expression was detected in 4% paraformaldehyde-fixed CD4+CD25+ T cells using a FITC-labeled anti-FoxP3 antibody. Similar results were obtained in 4 separate experiments.

FIGURE 7. CD4+CD25+ T cells isolated using EasySep™ are able to suppress T cell proliferation responses.



EasySep™ isolated CD4+CD25+ T cells were co-cultured at a ratio of 1:1 and 1:3 with stimulated CD4+CD25neg T cells to determine their ability to suppress T cell proliferation. Results are expressed relative to the level of proliferation detected in stimulated CD4+CD25neg cell cultures. The bars are the means of 4 experiments with error bars indicating standard deviations.

Flexible
Cell Isolation

Pre-Enrichment Kits

When purities of over 90% are required, STEMCELL's Pre-Enrichment Kits are an ideal way to save hours on flow sorting while getting purities you need for that crucial experiment. RosetteSep™ or EasySep™ Pre-Enrichment Kits provide excellent yields of pre-enriched Tregs from whole blood, buffy coat or PBMC, and allow you to go from sample to sorting in less than 90 minutes.

STARTING SAMPLE	PRODUCT	CATALOG #
Whole blood	RosetteSep™ Human CD4$^+$ T Cell Enrichment Cocktail	15062
Whole blood	RosetteSep™ Human CD4$^+$CD127^{low} T Cell Enrichment Cocktail	15361
PBMC	EasySep™ Human CD4$^+$CD127^{low} T Cell Enrichment Kit	19231
PBMC	EasySep™ Human CD4$^+$ T Cell Enrichment Kit	19052
Single cell suspensions	EasySep™ Mouse CD4$^+$ T Cell Pre-Enrichment Kit	19772

Customizable Cell Isolation

STEMCELL's Treg isolation kits are easily customizable. If you can't find the kit you need, we can help you find the antibodies and method to isolate the Treg subtype that you want. We have a team of technical experts who will work with you to help you develop the right cell isolation solution to meet your Treg research needs.

Did you know?

STEMCELL has made-to-order kits for the isolation of Tregs from rat and non-human primates. Contact techsupport@stemcell.com for more information.

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Complete
Product Listing

Cell Isolation Kits

SPECIES	STARTING SAMPLE	PHENOTYPE OF CELLS	PRODUCT	CATALOG #	PAGE
Human	Whole blood	$CD4^+CD25^+$ T cells	Complete Kit for Human $CD4^+CD25^+$ T Cells	15862	8
		$CD4^+CD127^{low}CD25^+$ T cells	Complete Kit for Human $CD4^+CD127^{low}CD25^+$ Regulatory T Cells	15861	9
		$CD4^+CD127^{low}CD49d^-CD25^+$ T cells	Complete Kit for Human $CD4^+CD127^{low}CD49d^-CD25^+$ Regulatory T Cells	15864	9
	PBMC	$CD4^+CD25^+$ T cells	EasySep™/RoboSep™ Human $CD4^+CD25^+$ T Cell Isolation Kit	18062	8
		$CD4^+CD127^{low}$ T cells	EasySep™/RoboSep™ Human $CD4^+CD127^{low}$ T Cell Enrichment Kit	19231	10
		$CD4^+CD127^{low}CD49d^-$ T cells	EasySep™/RoboSep™ Human $CD4^+CD127^{low}CD49d^-$ Regulatory T Cell Enrichment Kit	19232	10
Mouse	Spleen or other tissues	$CD4^+CD25^+$ T cells	EasySep™/RoboSep™ Mouse $CD4^+CD25^+$ T Cell Isolation Kit	19782	12

Pre-Enrichment Kits

SPECIES	STARTING SAMPLE	PRODUCT	CATALOG #	PAGE
Human	Whole blood	RosetteSep™ Human $CD4^+$ T Cell Enrichment Cocktail	15062	13
		RosetteSep™ Human $CD4^+CD127^{low}$ T Cell Enrichment Cocktail	15361	13
	PBMC	EasySep™ Human $CD4^+CD127^{low}$ T Cell Enrichment Kit	19231	13
		EasySep™ Human $CD4^+$ T Cell Enrichment Kit	19052	13
Mouse	Spleen or other tissues	EasySep™ Mouse $CD4^+$ T Cell Enrichment Kit	19772	13

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