CELL SEPARATION PRODUCTS FOR AUTOIMMUNITY RESEARCH

PRODUCTS FOR **AUTOIMMUNITY RESEARCH**

CELL TYPE	METHOD OF SELECTION	HUMAN			MOUSE
		ROSETTESEP	EASYSEP		EASYSEP
		WHOLE BLOOD	WHOLE BLOOD	PMBC[1]	SPLEEN[2]
T CELLS	Positive (CD3+) Positive (CD2+) Positive (CD90.1/ Thy1.2)		18081 18687	18051 18657	18751
	Negative	15021		19051	19751
	Depletion	15621			
CD4+ T CELLS	Positive (CD4+)		18082	18052	18752
	Negative	15022		19052	19752
	Depletion	15622			
CD8+ T CELLS	Positive (CD8+)		18083	18053	18753
	Negative	15023		19053	19753
	Depletion	15623			
REGULATORY CD4+CD25+ T CELLS	Positive	15862			19782
NAÏVE CD4+ T CELLS	Negative			19155	
MEMORY CD4+ T CELLS	Negative			19157	
B CELLS	Positive (CD19+)		18084	18054	18754
	Negative Negative (without CD43 Depletion)	15024		19054 19154	19754
PLASMA CELLS	Positive (CD138+)		18387	18357	
	Negative	15129			
LYMPHOID CELLS	Positive (CD3/CD19)		18684		
	Negative	15223			
NK CELLS	Positive (CD56) Positive (CD49b/DX5)		18085	18055	18755
	Negative	15025		19055	19755
DENDRITIC CELLS	Negative			19251	18768
PLASMACYTOID DENDRITIC CELLS	Negative			19062[3]	
MYELOID CELLS	Positive (CD15+) Positive (CD33+) Positive (CD33/66b)		18681 18287 18683	18257 18653	
MONOCYTES	Positive (CD14+)		18088	18058	
	Negative Negative (without CD16 Depletion)	15028		19059 19058	19761[3]
GRANULOCYTES	Positive (CD66b)		18682		
	Negative	15121			
	Depletion	15624			
NEUTROPHILS	Negative			19257	
EOSINOPHILS	Negative			19256	
BASOPHILS	Negative			19069	

1. Peripheral Blood Mononuclear Cells
2. Spleen or single cell suspension from any tissue.
3. Coming Soon!

OBTAIN FUNCTIONAL CELLS FOR **AUTOIMMUNITY RESEARCH**

In order to study the pathogenesis of autoimmune diseases, it is ideal to begin with highly viable functional cells. This requires cell isolation systems that are **gentle** to avoid damage or activation of cells. Many current cell isolation methods involve passing cells through columns, tubings, or flow cells that may unnecessarily activate them.

STEMCELL Technologies offers optimized cell isolation systems that isolate **"untouched" cells** which remain in suspension in a test tube throughout the cell isolation procedure. Isolated cells are thus viable and functional, making them ideal for studying cell activation, regulation, and suppression for autoimmunity research. A list of **functional data references** for isolated cells used in **rheumatoid arthritis, diabetes, multiple sclerosis,** and **systemic lupus erythematosus** research can be found on pages 10 - 11.

Optimized reagents and protocols for isolation of **Human** (see page 9) or **Mouse cells** (see page 8) from virtually **any source** such as whole blood, peripheral blood mononuclear cells, or single cell suspensions from tissues include:

T cells, $CD4^+$ T cells, $CD8^+$ T cells, Regulatory $CD4^+CD25^+$ T cells, Naïve or Memory $CD4^+$ T cells, B cells, Plasma cells, Dendritic Cells, Monocytes, Granulocytes (Basophils, Eosinophils, Neutrophils) , NK cells, and more (see page 2).

ADVANTAGES OF OUR CELL ISOLATION PRODUCTS:

- **Fast, easy, and simple protocols**
- **Untouched highly purified cells**
- **Functional cells immediately available for downstream assays**

CELL SEPARATION TECHNOLOGIES

RosetteSep®

RosetteSep® is a **rapid** cell separation procedure for the isolation of highly purified cells directly from **whole blood**. RosetteSep® turns a simple density centrifugation step into a specific antibody-mediated cell enrichment procedure (see Figure 1). **Desired cells** are **never labeled with antibody** and are **ready** for **functional assays**.

ADVANTAGES:

Simple. No special equipment required except a centrifuge.

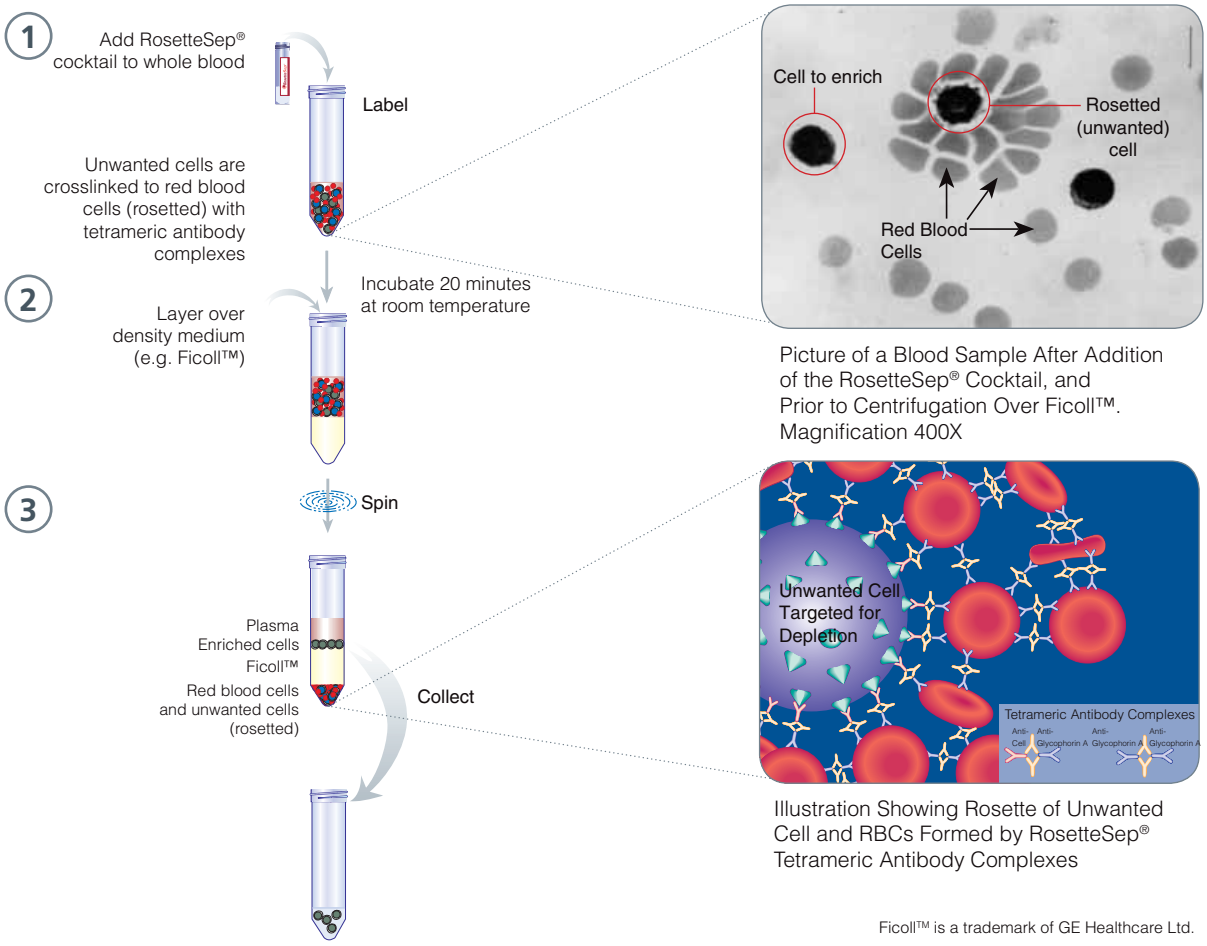
Fast. Just a 20 minute incubation at room temperature prior to a standard Ficoll spin (see Figure 1).

Easy. One-step cell enrichment directly from whole blood.

High Recovery. No post-Ficoll cell loss.

Untouched Cells. Cells are unlabeled and are immediately ready for functional assays.

FIGURE 1: RosetteSep® Procedure



Picture of a Blood Sample After Addition of the RosetteSep® Cocktail, and Prior to Centrifugation Over Ficoll™. Magnification 400X

Illustration Showing Rosette of Unwanted Cell and RBCs Formed by RosetteSep® Tetrameric Antibody Complexes

Ficoll™ is a trademark of GE Healthcare Ltd.

EasySep®

EasySep® is a **powerful immunomagnetic cell separation system** that can isolate a wide variety of cell types from virtually any source including peripheral blood mononuclear cells (PBMC), whole blood, and single cell suspensions from tissues. Cell separation can be performed manually using EasySep® or fully automated using **RoboSep®**, the only fully automated cell separator.

ADVANTAGES:

Simple. No columns or washes required (see Figure 2).

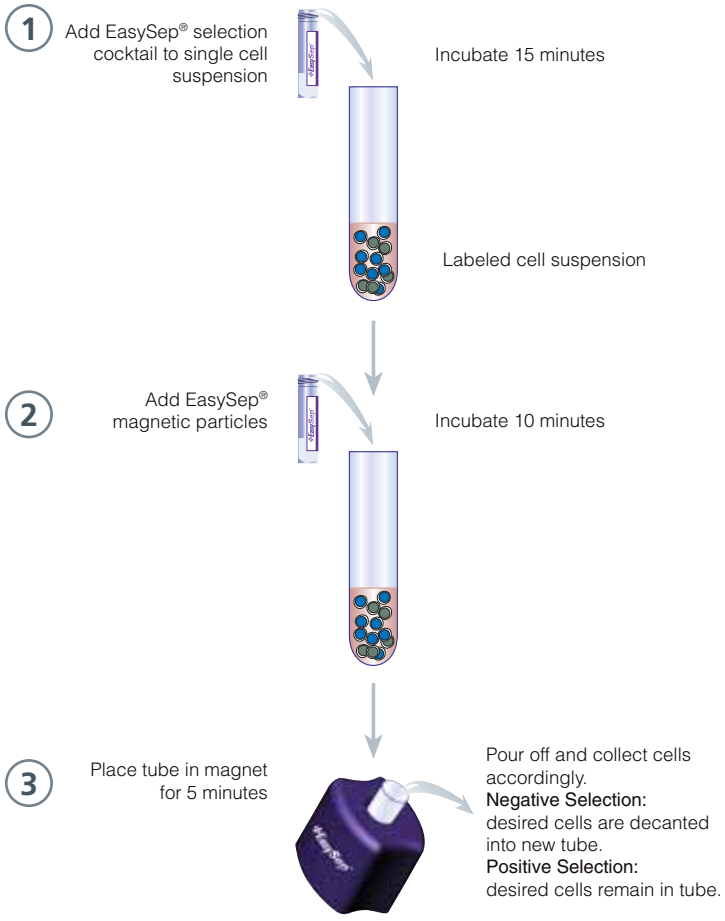
Gentle on Cells. Cells remain in solution eliminating the risk of mechanical damage.

Functional Cells. Untouched cells immediately ready for use in downstream assays.

High Purity. Purities of up to 99%.

Wide Range of Kits. A variety of specific cell types available (see page 2).

FIGURE 2: EasySep® Procedure



CELL SEPARATION TECHNOLOGIES

ROBOSEP®

RoboSep® uses a robotic pipettor to **perform all EasySep® cell labeling and magnetic separation steps**. RoboSep® set-up is simple - load your samples and reagents and return to separated cells in as little as 25 minutes.

ADVANTAGES:

Easy. Simply load your samples and walk-away.

High Capacity. Process up to 4 different cell types at once to isolate high numbers of cells.

Minimize sample handling. Only 5 minutes of "hands-on" time is required per run.

Eliminate Sample Contamination. Uses disposable tips – instead of columns or tubing.

Walk Away Automation. RoboSep® is the only cell separator that fully automates all cell labeling and separation steps to save technician time.

FIGURE 3: RoboSep® Procedure

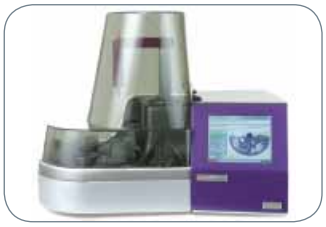
1

Select protocol. Load samples, EasySep® Selection Cocktail, magnetic particles, buffer and tips in carousel.

2

Press Run.

3



RoboSep® processes samples (approx. 25 - 60 min/run).

4

Return to collect your separated cells.

“ We like the reliability of the RoboSep®,

the minimization/elimination of specimen handling by the tech during subset separation, and the low maintenance of the instrument. These factors are important to us with such a high throughput of samples processed. ”

Wendy Leong, LAB MANAGER
PATHOLOGY/BLOOD CENTER LABORATORY

“ We get up to eight samples a day that we sort for CD138+ cells. These cells are for different studies at the clinic and have to be processed the same day. Using our old system it would take up to 30 minutes per sample because of the volume and we’d have to be there all the time to load, unload and clean the machine. Now we can run four samples at once and have our enriched cells in an hour. RoboSep® uses dedicated pipette tips for each sample so there’s no issue with cross-contamination. Because the process is all automated we can get other things done in the meantime. It makes our job a lot easier when we don’t have to check the timer every ten minutes.

We’re a busy lab and RoboSep® really saves us a lot of time. ”

Kim Henderson, RESEARCHER
CLINICAL LAB IN THE MIDWESTERN UNITED STATES

ROBOSEP®
THE FULLY AUTOMATED CELL SEPARATOR

MOUSE CELL SEPARATION FOR AUTOIMMUNITY RESEARCH

STEMCELL offers a wide variety of kits for the isolation of mouse cells for autoimmunity research. Starting with single cell suspensions of spleen or tissues (i.e. lymph nodes, bone marrow, etc.), specific cell types from mice can be highly purified using EasySep® Mouse kits.

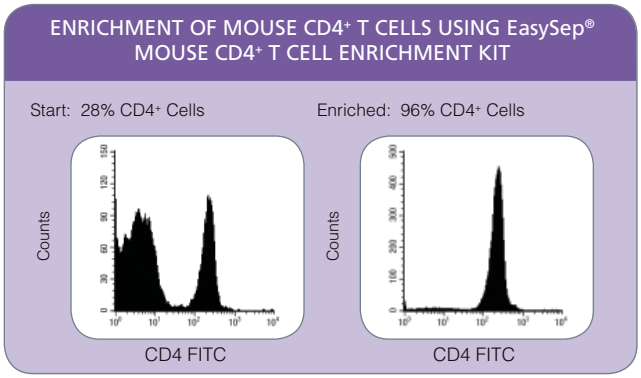
EasySep® MOUSE KITS AVAILABLE FOR THE POSITIVE AND NEGATIVE SELECTION OF:

- **T cells**
- **CD4⁺ T cells**
- **Regulatory CD4⁺CD25⁺ T cells**
- **CD8⁺ T cells**
- **B cells**
- **NK cells**
- **Dendritic cells**

See page 2 for more cell types.

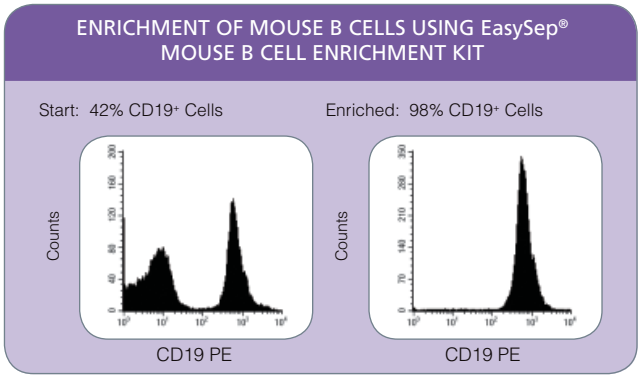
TYPICAL PERFORMANCE DATA:

FIGURE 4: EasySep® CD4⁺ T Cell Enrichment Kit (Catalog #19752)



Starting with mouse splenocytes, the CD4⁺ cell content of the enriched fraction typically ranges from 94 - 96%.

FIGURE 5: EasySep® B Cell Enrichment Kit (Catalog #19754)



Starting with mouse splenocytes, the CD19⁺ cell content of the enriched fraction typically ranges from 97 - 99%.

HUMAN CELL SEPARATION FOR AUTOIMMUNITY RESEARCH

Optimized Human RosetteSep® or EasySep® kits are available for the isolation of human **T cells, CD4⁺ T cells, CD8⁺ T cells, B cells, Plasma cells, Monocytes, Dendritic cells, Granulocytes, NK cells** and more (see page 2).

In addition, **Regulatory CD4⁺CD25$^{+bright}$ T cells (Catalog #15862)** can now be isolated in a simple 2-step procedure by combining **RosetteSep® CD4⁺ T Cell Enrichment** with **EasySep® Positive Selection of CD25$^{+bright}$** cells. Highly purified Regulatory T cells can be enriched in less than two hours.

FUNCTIONAL STUDIES SHOW THAT ISOLATED REGULATORY CD4⁺CD25$^{+BRIGHT}$ T CELLS:

- Efficiently suppress CD25^{neg} T cell proliferation in response to CD3/CD28 stimulation (see Figure 6)
- Express the regulatory T cell-specific FOXP3 transcription factor at high levels (see Figure 7)
- Express CD62L, GITR, CTLA, and HLA-DR (see STEMCELL's Regulatory T Cell Technical Note, Catalog #29147)

Data obtained from collaboration with Dr. Megan Levings (Dept. of Surgery, University of British Columbia) and Dr. Rajendra Pahwa (Diabetes Research Institute, University of Miami Miller School of Medicine).

FIGURE 6: Isolated CD4⁺CD25⁺ T cells are anergic and suppress proliferation of CD4⁺CD25^{neg} T cells

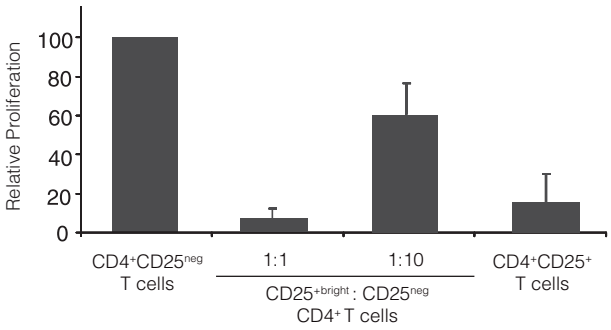
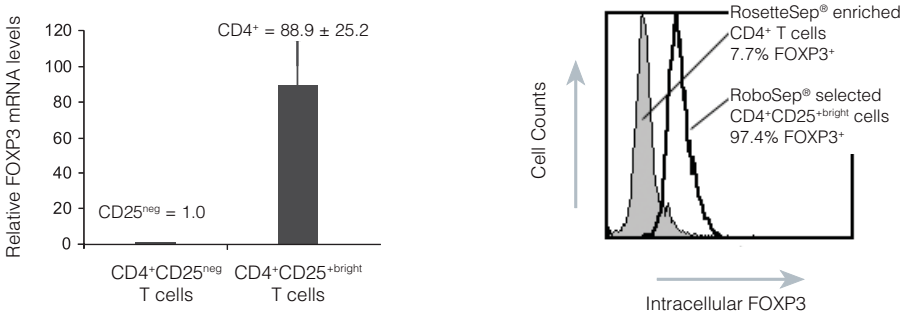


FIGURE 7: FOXP3 measurements in isolated CD4⁺ T cell populations



FUNCTIONAL DATA REFERENCES:

T CELLS:

Stimulation of **T cell** autoreactivity by anomalous expression of NKG2D and its MIC ligands **in rheumatoid arthritis**. Veronika Groh, Anja Brühl, Hani El-Gabalawy, J. Lee Nelson, and Thomas Spies. PNAS, Aug 2003; 100: 9452 - 9457.

Phosphorylated ERM Is Responsible for Increased **T Cell** Polarization, Adhesion, and Migration in Patients with **Systemic Lupus Erythematosus**. Yansong Li, Tatsuhiro Harada, Yuang-Taung Juang, Vasileios C. Kyttaris, Ying Wang, Michael Zidanic, Kenneth Tung, and George C. Tsokos. J. Immunol., Feb 2007; 178: 1938 - 1947.

Among CXCR3 Chemokines, IFN-γ Inducible Protein of 10 kDa (CXC Chemokine Ligand (CXCL) 10) but Not Monokine Induced by IFN-γ (CXCL9) Imprints a Pattern for the Subsequent Development of **Autoimmune Disease**. Urs Christen, Dorian B. McGavern, Andrew D. Luster, Matthias G. von Herrath, and Michael B. A. Oldstone. J. Immunol., Dec 2003; 171: 6838 - 6845.

Aberrant expression of BAFF in **T cells** of **systemic lupus erythematosus**, which is recapitulated by a human T cell line. Loucy Keiko Yoshimoto, Yasue Takahashi, Mie Ogasawara, Yumiko Setoyama, Katsuya Suzuki, Kensei Tsuzaka, Tohru Abe, and Tsutomu Takeuchi. Int. Immunol., Jul 2006; 18: 1189 - 1196.

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Natural Naive $CD4^+CD25^+CD127^{low}$ **Regulatory T Cell** (Treg) Development and Function Are Disturbed in **Multiple Sclerosis Patients**: Recovery of Memory Treg Homeostasis during Disease Progression. Koen Venken, Niels Hellings, Tom Broekmans, Karen Hensen, Jean-Luc Rummens, and Piet Stinissen. J. Immunol., May 2008; 180: 6411 - 6420.

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Novel Immunomodulatory Properties of Berbamine through Selective Down-Regulation of STAT4 and Action of IFN-γ in **Experimental Autoimmune Encephalomyelitis**. Yiping Ren, Limin Lu, Taylor B. Guo, Ju Qiu, Yiqing Yang, Ailian Liu, and Jingwu Z. Zhang. J. Immunol., Jul 2008; 181: 1491 - 1498.

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CD40 ligand and MHC class II expression are essential for human peripheral **B cell tolerance**. Maxime Hervé, Isabelle Isnardi, Yen-shing Ng, James B. Bussel, Hans D. Ochs, Charlotte Cunningham-Rundles, and Eric Meffre. J. Exp. Med., Jul 2007; 204: 1583 - 1593.

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