## PRODUCTS FOR AUTOIMMUNITY RESEARCH

<table>
<thead>
<tr>
<th>CELL TYPE</th>
<th>METHOD OF SELECTION</th>
<th>HUMAN ROSETTESEP</th>
<th>EASYSEP Whole Blood</th>
<th>EASYSEP PMBC</th>
<th>MOUSE EASYSEP Spleen</th>
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<td><strong>T CELLS</strong></td>
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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
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

1. Add RosetteSep® cocktail to whole blood
2. Layer over density medium (e.g. Ficoll™) at room temperature
3. Spin

Unwanted cells are crosslinked to red blood cells (rossetted) with tetrameric antibody complexes.

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® 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**

1. **Add EasySep® selection cocktail to single cell suspension**
   - Incubate 15 minutes
   - Labeled cell suspension

2. **Add EasySep® magnetic particles**
   - Incubate 10 minutes

3. **Place tube in magnet for 5 minutes**
   - Pour off and collect cells accordingly.
   - **Negative Selection:** desired cells are decanted into new tube.
   - **Positive Selection:** desired cells remain in tube.
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
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)

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

**ENRICHMENT OF MOUSE CD4+ T CELLS USING EasySep® MOUSE CD4+ T CELL ENRICHMENT KIT**

- Start: 28% CD4+ Cells
- Enriched: 96% CD4+ Cells

**ENRICHMENT OF MOUSE B CELLS USING EasySep® MOUSE B CELL ENRICHMENT KIT**

- Start: 42% CD19+ Cells
- Enriched: 98% CD19+ Cells

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

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 CD25neg 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+CD25neg T cells

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

T CELLS:


T CELL SUBSETS:


**B CELLS:**


**OTHER CELLS:**

A rapid two-step procedure for the purification of human peripheral blood basophils to near homogeneity. Gibbs BF, Papenfuss K. and Falcone FH. Clinical and Experimental Allergy, Mar 2008; 38 : 480 - 5

