Fast and Easy Isolation Of

B Cells

STEMCELL Technologies
Table of Contents

3 Cutting-Edge B Cell Research
4 Isolate B Cells In As Little As 25 Minutes
5 Research and Applications
6 EasySep™: Immunomagnetic B Cell Isolation Without Columns
6 RoboSep™: Fully Automated B Cell Isolation
7 RosetteSep™: One-Step Untouched B Cell Isolation From Whole Blood
8 Human B Cells: Starting with Whole Blood
9 Human B Cells: Starting with PBMC or Leukapheresis Samples
10 Human Naive and Memory B Cells: Starting with PBMC or Leukapheresis Samples
11 Human CD138⁺ Plasma Cells: Starting with PBMC, Whole Blood or Bone Marrow
12 Mouse B Cells: Starting with Spleen or Other Tissues
13 Related Products
14 Product Listing
15 References
B cells express cell surface immunoglobulin receptors that recognize specific antigenic epitopes, and are a pivotal component of the adaptive immune system and in particular, humoral immune responses. B cells mediate many processes necessary for immune homeostasis including antibody production, antigen presentation, cytokine secretion, T cell co-stimulation, and tumor immunity. Conversely, their dysregulation is the basis of several immune pathologies including autoimmunity, leukemia / lymphoma, and multiple myeloma.

Isolation of highly purified B cells is the first step towards successful B cell research. Isolate whole B cell populations as well as various B cell subsets directly from human whole blood, buffy coat, leukapheresis samples, or PBMC, and from mouse spleen or other tissues, with high purity and recovery using the fast and easy B cell isolation kits from STEMCELL Technologies.

With the unique cell separation platforms EasySep™, RosetteSep™, and RoboSep™, STEMCELL Technologies is poised to support your immunological research endeavors.
Isolate B Cells In As Little As 25 Minutes
With High Purity And Preserved Viability

Fast and Easy
Magnetic or immunodensity separation without columns

High Purity and Recovery
Get high recovery, and purity of up to 99% pure B cells

B Cells are Immediately Ready for Use
Magnetic particles do not interfere with downstream applications such as flow cytometry

B Cells Retain Functionality
Isolated B cells have been used in various scientific investigations, including publications that:
- Identify intermediate cell populations in naïve B cell development¹
- Investigate modulators of human B cell differentiation²,³
- Demonstrate the role of innate immunity signaling pathways in B cell tolerance⁴
- Characterize the mechanism of plasma and memory B cell selection⁵
- Evaluate gene expression in B cell subsets⁶
Research and Applications
Isolated B Cells Are Suitable For All Downstream Analysis

The gentle, column-free cell separation platforms RosetteSep™, EasySep™ and RoboSep™ each preserve B cell viability, ensuring that purified B cells are suitable for functional and biological studies. Both enriched and positively selected B cells are immediately ready for downstream assays. Negatively enriched B cells are untouched by EasySep™ reagents. Positively selected B cells are labeled with small EasySep™ magnetic particles that are flow cytometry compatible. B cells can be routinely and reliably isolated at up to 99% purity, making them highly complementary to molecular diagnostic techniques used for hematologic malignancy analysis.

B Cell Functionality
Isolated B cells are highly functional and have been used for:
- Lipid antigen presentation to NKT cells
- Intracellular calcium flux assays
- Hybridoma generation
- Adoptive transfer

HLA Testing
The HLA B cell isolation products are specifically optimized for isolating B cells for HLA testing purposes and have been formulated for:
- Serology-based assays
- Flow Cytometry Crossmatch (FCXM)
- Chimerism analysis

Hematologic Malignancies
Purifying malignant B cells can greatly improve the accuracy and reliability of molecular diagnostic studies. Isolate plasma cells and B cells for the study of:
- Multiple Myeloma
- Chronic Lymphocytic Leukemia
EasySep™

Immunomagnetic B Cell Isolation Without Columns

EasySep™ is a fast and easy way to get highly purified B cells from whole blood, buffy coat, fresh or previously frozen peripheral blood mononuclear cells (PBMC), leukapheresis samples, and mouse spleen or other tissue samples. Cells are crosslinked to EasySep™ magnetic particles using the Tetrameric Antibody Complex (TAC) technology, and then separated from unwanted cells with an EasySep™ magnet without the use of columns.

EasySep™ Human B Cell Enrichment Procedure

1. Add EasySep™ selection cocktail to single cell suspension
2. Incubate 5 minutes
3. Place tube in magnet for 2.5 minutes
4. Incubate 10 minutes
5. Pour off supernatant containing untouched B cells into a new tube.

FIGURE 1. Tetrameric Antibody Complex (TAC) crosslinking a cell to a dextran coated magnetic particle.

RoboSep™

Fully Automated B Cell Isolation

Streamline your B cell separations using RoboSep™, the fully automated cell separator. By performing all EasySep™ cell labeling and separation steps, RoboSep™ maintains the speed and simplicity of EasySep™ while offering walk-away automation, enabling the high-throughput, versatile isolation of highly purified cells. Minimize sample handling and eliminate cross-contamination while isolating B cells with just 5 minutes of “hands-on” technician time.
RosetteSep™
One-Step Untouched Human B Cell Isolation From Whole Blood

RosetteSep™ is a rapid immunodensity procedure for the isolation of untouched B cells directly from whole blood. It eliminates the need for a separate magnetic separation step by isolating cells during a standard density centrifugation Ficoll™ step, significantly reducing handling time and maximizing convenience. RosetteSep™ crosslinks unwanted cells to red blood cells present in the sample to form immunorosettes. When centrifuged over a density medium, the unwanted cells pellet along with the red blood cells, leaving desired cells at the interface between the plasma and the density medium.

Did You Know?
Although RosetteSep™ has been optimized for use with whole blood, cells can be enriched from other sources (for example, buffy coat or leukapheresis samples.) The concentration of nucleated cells in the sample should not exceed 5 x 10⁷ cells/mL, and red blood cells (RBCs) should be present at a ratio of at least 30 - 50 RBCs per nucleated cell.

Ficoll™ and Ficoll-Paque™ are trademarks of GE Healthcare Ltd.
Human B Cells
Starting With Whole Blood

Isolate untouched human B cells by negative selection, or positively select CD19⁺, CD20⁺, or CD19⁺/CD20⁺ cells directly from whole blood.

RosetteSep™ Human B Cell Enrichment Cocktail (Catalog #15024)

Isolate untouched human B cells directly from whole blood without magnets or special equipment.

HOW IT WORKS:
The B cell population is enriched by negative selection using RosetteSep™, which depletes unwanted cells and leaves the desired B cells untouched by antibody.

Typical FACS Histogram Results with RosetteSep™ Human B Cell Enrichment Cocktail
Start: 6% CD19⁺ cells	 Enriched: 81% CD19⁺ cells

Starting with fresh whole blood, the CD19⁺ cell content of the enriched fraction typically ranges from 81 - 83%.

This cocktail has been used to isolate B cells for:
- Intracellular calcium flux assays
- Immunofluorescence and FISH analysis
- Chromatin immunoprecipitation analysis

EasySep™ Human Whole Blood CD19 Positive Selection Kit (Catalog #18084)

Isolate CD19⁺ human B cells directly from whole blood.

HOW IT WORKS:
CD19⁺ B cells are isolated by positive selection using EasySep™.

Typical FACS Histogram Results with EasySep™ Human Whole Blood CD19 Positive Selection Kit.
Start: 2% CD19⁺ cells	 Selected: 99% CD19⁺ cells

Starting with fresh whole blood, the CD19⁺ cell content of the selected fraction typically ranges from 94 - 99%.

This kit has been used to isolate B cells for:
- Hybridoma generation
- Therapeutic monoclonal antibody production
- Hybridoma optimization using morphogenics

Also Available
EasySep™ Human Whole Blood CD20 Positive Selection Kit (Catalog #18685)
Isolate CD20⁺ B cells directly from whole blood. Starting with fresh whole blood, the CD20⁺ cell content of the selected fraction typically ranges from 96 - 99%.

B Cell Kits for HLA Analysis
See page 14.
**Human B Cells**

Starting With PBMC Or Leukapheresis Samples

Isolate untouched human B cells (with or without CD43 depletion) by negative selection directly from PBMC or leukapheresis samples.

**EasySep™ Human B Cell Enrichment Kit (Catalog #19054)**

Isolate untouched B cells from PBMC or leukapheresis samples.

**HOW IT WORKS:**
The B cell population is enriched by negative selection using EasySep™, which depletes unwanted cells and leaves desired B cells untouched by antibody.

**Typical FACS Histogram Results with EasySep™ Human B Cell Enrichment Kit**

<table>
<thead>
<tr>
<th>Start</th>
<th>Enriched</th>
</tr>
</thead>
<tbody>
<tr>
<td>8% CD19+ cells</td>
<td>99% CD19+ cells</td>
</tr>
</tbody>
</table>

Starting with fresh or previously frozen PBMC, the CD19+ cell content of the enriched fraction typically ranges from 95 - 99%.

This kit has been used to isolate B cells for:
- Lipid antigen presentation to NKT cells
- DNAse protection assays
- Gene expression profiling by microarray analysis

**EasySep™ Human B Cell Enrichment Kit without CD43 Depletion (Catalog #19154)**

Isolate untouched human B cells from PBMC samples that include B cells that express CD43 (e.g. CLL samples).

**HOW IT WORKS:**
The B cell population, including CD43+ B cells, is enriched by negative selection using EasySep™, which depletes unwanted cells and leaves desired B cells untouched by antibody.

**Typical FACS Histogram Results with EasySep™ Human B Cell Enrichment Kit (without CD43 Depletion)**

<table>
<thead>
<tr>
<th>Start</th>
<th>Enriched</th>
</tr>
</thead>
<tbody>
<tr>
<td>11% CD19+ cells</td>
<td>96% CD19+ cells</td>
</tr>
</tbody>
</table>

Starting with fresh or previously frozen PBMC, the CD19+ cell content of the enriched fraction typically ranges from 87 - 98%.

This kit has been used to isolate B cells for:
- Quantitative rt-PCR analysis in Chronic Lymphocytic Leukemia (CLL)
- Analyzing the specificity of cytotoxic drugs for CLL
- Gene expression profiling in CLL

**Also Available**

**EasySep™ Human CD19 Positive Selection Kit (Catalog #18054)**
Isolate CD19+ B cells from PBMC. Starting with fresh or previously frozen PBMC, the CD19+ cell content of the selected fraction typically ranges from 97 - 99%.

**B Cell Kits for HLA Analysis**
See page 14.
Isolate specific human B cell subsets from PBMC or leukapheresis samples. Isolate untouched naïve B cells by negative selection or positively select CD27⁺ memory B cells from a pre-enriched B cell population.

**EasySep™ Human Naïve B Cell Enrichment Kit (Catalog #19254)**

Isolate untouched human naïve B cells from PBMC or leukapheresis samples.

**HOW IT WORKS:**
The naïve B cell population is enriched by negative selection using EasySep™ which depletes unwanted cells and leaves desired cells untouched by antibody.

**Typical FACS Profile Results with EasySep™ Human Naïve B Cell Enrichment Kit**

Start: 4% CD19⁺ CD27⁻ cells  
Enriched: 97% CD19⁺ CD27⁻ cells

Starting with fresh or previously frozen PBMC, the CD19⁺CD27⁻ cell content of the enriched fraction typically ranges from 92 - 98%.

**EasySep™ Human Memory B Cell Isolation Kit (Catalog #18164)**

Isolate human memory B cells from PBMC or leukapheresis samples.

**HOW IT WORKS:**
The B cell population is first enriched by negative selection. CD27⁺ memory B cells are then isolated using EasySep™ CD27 positive selection.

**Typical FACS Profile Results with EasySep™ Human Memory B Cell Isolation Kit**

Start: 6% CD19⁺ CD27⁺ cells  
Selected: 92% CD19⁺ CD27⁺ cells

Starting with fresh or previously frozen PBMC, the CD19⁺CD27⁺ cell content of the enriched fraction typically ranges from 85 - 95%.

**Did You Know?**

We also provide comprehensive protocols on how to isolate highly purified naïve and memory B cells from the same sample. Contact techsupport@stemcell.com for a copy.
Human CD138⁺ Plasma Cells
Starting with PBMC, Whole Blood or Bone Marrow

Isolate CD138⁺ (normal and malignant) plasma cells directly from bone marrow, PBMC, or whole blood.

**EasySep™ Human CD138 Positive Selection Kit (Catalog #18357)**

Isolate CD138⁺ plasma cells from PBMC or bone marrow.

**HOW IT WORKS:**
CD138⁺ (Syndecan-1) cells are isolated by positive selection using EasySep™. CD138 is expressed on normal or malignant plasma cells, but not on mature B cells. Isolated cells are highly suited for multiple myeloma research.

![Typical FACS Histogram Results with EasySep™ Human CD138 Positive Selection Kit](chart1)

- Start: 10% CD138⁺ cells
- Selected: 91% CD138⁺ cells

Starting with fresh or previously frozen PBMC, the CD138⁺/Syndecan-1 cell content of the selected fraction typically ranges from 85 - 95%. Please note that purity is highly dependent on the starting sample.

**EasySep™ Human Whole Blood CD138 Positive Selection Kit (Catalog #18387)**

Isolate CD138⁺ plasma cells from whole blood.

**HOW IT WORKS:**
CD138⁺ (Syndecan-1) cells are isolated by positive selection using EasySep™. CD138 is expressed on normal or malignant plasma cells, but not on mature B cells. Isolated cells are highly suited for multiple myeloma research.

![Typical FACS Histogram Results with EasySep™ Human Whole Blood CD138 Positive Selection Kit](chart2)

- Start: 6% CD138⁺ cells
- Selected: 94% CD138⁺ cells

Starting with fresh whole blood spiked with a multiple myeloma cell line, the CD138⁺/Syndecan-1 cell content of the selected fraction typically ranges from 89 - 98%.

*Residual red blood cells were removed by lysis prior to flow cytometry.

These kits have been used to isolate plasma cells for research of multiple myeloma17, primary systemic amyloidosis18 and plasma cell leukemia19.

**Also Available**

**RosetteSep™ Human Multiple Myeloma Cell Enrichment Cocktail (Catalog #15129)**

Isolate untouched multiple myeloma cells (B cells and plasma cells) from fresh human bone marrow aspirates by negative selection, without the use of magnets or special equipment.
Isolate untouched mouse B cells by negative selection, or positively select CD19+ B cells directly from single cell suspensions of spleen or other tissues.

**EasySep™ Mouse B Cell Enrichment Kit (Catalog #19754)**

Isolate untouched mouse B cells from single cell suspensions of spleen or other tissues.

**HOW IT WORKS:**
The B cell population is enriched by negative selection using EasySep™ which depletes unwanted cells and leaves desired cells untouched by antibody.

![Typical FACS Histogram Results with EasySep™ Mouse B Cell Enrichment Kit](chart.png)

Starting with mouse splenocytes, the CD19+ cell content of the enriched fraction typically ranges from 91 - 96%.

This kit has been used to isolate primary B cells for:
- Protein extraction and immunoblotting \(^{20,21}\)
- Analysis of class-switching recombination \(^{22,23}\)
- Retroviral transduction \(^{23}\)

**EasySep™ Mouse CD19 Positive Selection Kit (Catalog #18754)**

Isolate CD19+ mouse B cells from single cell suspensions of spleen or other tissues.

**HOW IT WORKS:**
CD19+ B cells are isolated by positive selection using EasySep™.

![Typical FACS Histogram Results with EasySep™ Mouse CD19 Positive Selection](chart.png)

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

This kit has been used to isolate primary B cells for:
- Co-culture with tumor cells \(^{24}\)
- Quantitative RT-PCR \(^{25}\)
- Adoptive Transfer \(^{10}\)
## Related Products

### Equipment

<table>
<thead>
<tr>
<th>PRODUCTS</th>
<th>DESCRIPTION</th>
<th>CATALOG #</th>
</tr>
</thead>
<tbody>
<tr>
<td>EasySep™ Magnet</td>
<td>Immunomagnetic Column-Free Magnet (for 5 mL tubes)</td>
<td>18000</td>
</tr>
<tr>
<td>“The Big Easy” EasySep™ Magnet</td>
<td>Immunomagnetic Column-Free Magnet (for 14 mL tubes)</td>
<td>18001</td>
</tr>
<tr>
<td>“Easy50” EasySep™ Magnet</td>
<td>Immunomagnetic Column-Free Magnet (for 50 mL tubes)</td>
<td>18002</td>
</tr>
<tr>
<td>EasySep™ Multistand</td>
<td>Holds up to 4 EasySep™ or 4 “The Big Easy” EasySep™ Magnets for separating up to 4 samples at once</td>
<td>18010</td>
</tr>
<tr>
<td></td>
<td>18010 with 4 EasySep™ Magnets</td>
<td>18004</td>
</tr>
<tr>
<td></td>
<td>18010 with 4 “The Big Easy” EasySep™ Magnets</td>
<td>18100</td>
</tr>
<tr>
<td>“EasyPlate” EasySep™ Magnet</td>
<td>Immunomagnetic Column-Free Magnet (holds a 96-well plate)</td>
<td>18102</td>
</tr>
<tr>
<td>RoboSep™</td>
<td>The Fully Automated Cell Separator</td>
<td>20000</td>
</tr>
</tbody>
</table>

### Antibodies

<table>
<thead>
<tr>
<th>PRODUCTS</th>
<th>DESCRIPTION</th>
<th>CATALOG #</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19 Antibody, Clone 4G7, PE-Conjugated</td>
<td>Mouse Monoclonal Antibody to Human CD19 - PE Conjugated</td>
<td>10509</td>
</tr>
<tr>
<td>CD19 Antibody, Clone 4G7, FITC-Conjugated</td>
<td>Mouse Monoclonal Antibody to Human CD19 - FITC Conjugated</td>
<td>10409</td>
</tr>
<tr>
<td>CD20 Antibody, Clone L27, PE-Conjugated</td>
<td>Mouse Monoclonal Antibody to Human CD20 - PE Conjugated</td>
<td>10510</td>
</tr>
<tr>
<td>CD20 Antibody, Clone L27, FITC-Conjugated</td>
<td>Mouse Monoclonal Antibody to Human CD20 - FITC Conjugated</td>
<td>10410</td>
</tr>
</tbody>
</table>
## Product Listing

### Complete Product Listing

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>STARTING SAMPLE</th>
<th>B CELL PHENOTYPE</th>
<th>PRODUCT</th>
<th>CATALOG #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Whole blood</td>
<td>All B cells</td>
<td>RosetteSep™ Human B Cell Enrichment Cocktail</td>
<td>15024</td>
</tr>
<tr>
<td></td>
<td>Multiple myeloma cells</td>
<td>RosetteSep™ Human Multiple Myeloma Cell Enrichment Cocktail</td>
<td>15129</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD19⁺</td>
<td>EasySep™/RoboSep™ Human Whole Blood CD19 Positive Selection Kit</td>
<td>18084/18084RF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD20⁺</td>
<td>EasySep™/RoboSep™ Human Whole Blood CD20 Positive Selection Kit</td>
<td>18685/18685RF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD138⁺ (plasma cells)</td>
<td>EasySep™/RoboSep™ Human Whole Blood CD138 Positive Selection Kit</td>
<td>18387/18387RF</td>
<td></td>
</tr>
<tr>
<td>PBMC</td>
<td>All B cells</td>
<td>EasySep™/RoboSep™ Human B Cell Enrichment Kit</td>
<td>19054/19054RF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>All B cells without CD43 depletion</td>
<td>EasySep™/RoboSep™ Human B Cell Enrichment Kit without CD43 Depletion</td>
<td>19154/19154RF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Naïve B cells</td>
<td>EasySep™/RoboSep™ Human Naïve B Cell Enrichment Kit</td>
<td>19254/19254RF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Memory B cells</td>
<td>EasySep™/RoboSep™ Human Memory B Cell Isolation Kit</td>
<td>18164/18164RF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD19⁺</td>
<td>EasySep™/RoboSep™ Human CD19 Positive Selection Kit</td>
<td>18054/18054RF</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CD138⁺ (plasma cells)</td>
<td>EasySep™/RoboSep™ Human CD138 Positive Selection Kit</td>
<td>18357/18357RF</td>
<td></td>
</tr>
<tr>
<td>Custom</td>
<td></td>
<td>EasySep™/RoboSep™ Human Custom Enrichment Kit</td>
<td>19309/19309RF</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EasySep™/RoboSep™ Human Custom Positive Selection Kit</td>
<td>18309/18309RF</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>Spleen or other tissue</td>
<td>All B cells</td>
<td>EasySep™/RoboSep™ Mouse B Cell Enrichment Kit</td>
<td>19754/19754RF</td>
</tr>
<tr>
<td></td>
<td>CD19⁺</td>
<td>EasySep™/RoboSep™ Mouse CD19 Positive Selection Kit</td>
<td>18754/18754RF</td>
<td></td>
</tr>
<tr>
<td>Custom</td>
<td></td>
<td>EasySep™/RoboSep™ Mouse Custom Enrichment Kit</td>
<td>19709/19709RF</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EasySep™/RoboSep™ Mouse Custom Positive Selection Kit</td>
<td>18709/18709RF</td>
<td></td>
</tr>
</tbody>
</table>

### B Cell Kits for HLA Analysis

<table>
<thead>
<tr>
<th>STARTING SAMPLE</th>
<th>B CELL PHENOTYPE</th>
<th>PRODUCT</th>
<th>CATALOG #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>All B cells</td>
<td>RosetteSep™ HLA B Cell Enrichment Cocktail</td>
<td>15064HLA</td>
</tr>
<tr>
<td></td>
<td>CD19⁺/CD20⁺</td>
<td>EasySep™/RoboSep™ HLA Whole Blood B Cell Positive Selection Kit</td>
<td>18184HLA/18184HLARF</td>
</tr>
<tr>
<td></td>
<td>CD19⁺</td>
<td>EasySep™/RoboSep™ Human Whole Blood CD19 Positive Selection Kit</td>
<td>18084/18084RF</td>
</tr>
<tr>
<td></td>
<td>CD20⁺</td>
<td>EasySep™/RoboSep™ Human Whole Blood CD20 Positive Selection Kit</td>
<td>18685/18685RF</td>
</tr>
<tr>
<td>PBMC</td>
<td>All B cells</td>
<td>EasySep™/RoboSep™ HLA B Cell Enrichment Kit</td>
<td>19054HLA/19054HLARF</td>
</tr>
<tr>
<td></td>
<td>CD19⁺/CD20⁺</td>
<td>EasySep™/RoboSep™ HLA B Cell Positive Selection Kit</td>
<td>18454HLA/18454HLARF</td>
</tr>
<tr>
<td></td>
<td>CD19⁺</td>
<td>EasySep™/RoboSep™ Human CD19 Positive Selection Kit</td>
<td>18054/18054RF</td>
</tr>
</tbody>
</table>
References


