Ready·Sep·Go

Fast and Easy Isolation Of B Cells

STEMCELL™ TECHNOLOGIES

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Cutting-Edge B Cell Research

Begins With Fast And Easy B Cell Isolation

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.

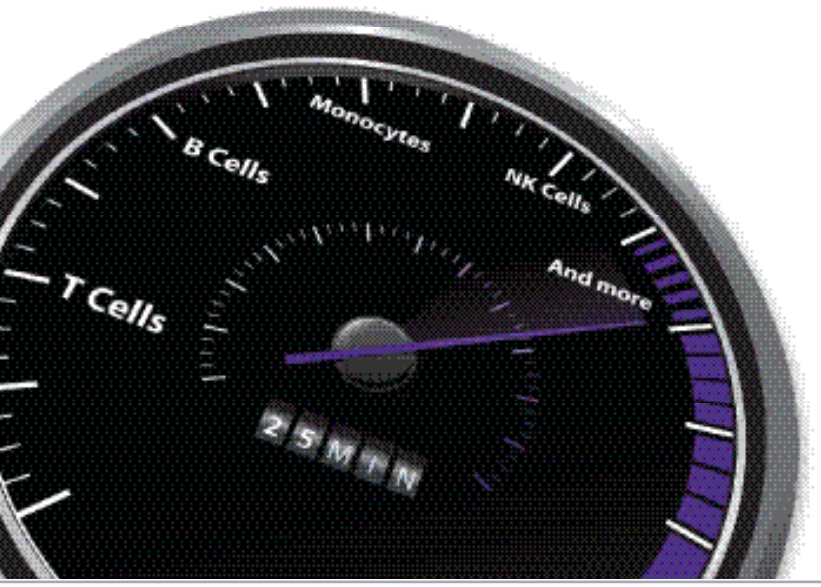
With the unique cell separation platforms **EasySep™**, **RosetteSep™** and **RoboSep™**, STEMCELL Technologies is poised to support your immunological research endeavors.

Ready·Sep·Go

Isolate B Cells In As Little As **25 Minutes**

Isolate whole B cell populations or specific B cell subsets in as little as 25 minutes using a B cell separation kit from our complete range.

- Whole B cell populations
- Naïve B cells
- Memory B cells
- CD138^{+} plasma cells
- B cells for CLL and multiple myeloma research
- B cells for HLA analysis
- Customized B cell isolations



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

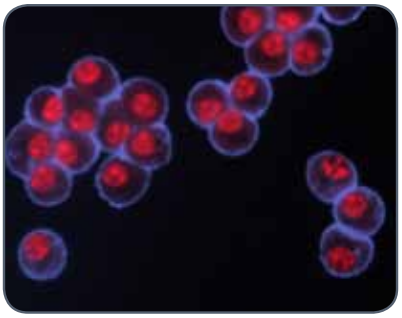
Isolated B cells have been used in various scientific investigations, including publications that:

- Identify intermediate cell populations in naïve B cell development[1]
- Investigate modulators of human B cell differentiation[2,3]
- Demonstrate the role of innate immunity signaling pathways in B cell tolerance[4]
- Characterize the mechanism of plasma and memory B cell selection[5]
- Evaluate gene expression in B cell subsets[6]

Research and Applications

Isolated B Cells Are Suitable For All Downstream Analysis

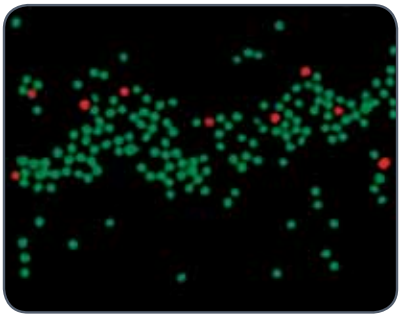
The gentle, column-free cell separation platforms **RosetteSep™**, **EasySep™** and **RoboSep™** each preserve B cell viability, ensuring that purified B cells are suitable for functional and biological studies. Both enriched and positively selected B cells are immediately ready for downstream assays. 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 hematalogic malignancy analysis.



B Cell Functionality

Isolated B cells are highly functional and have been used for:

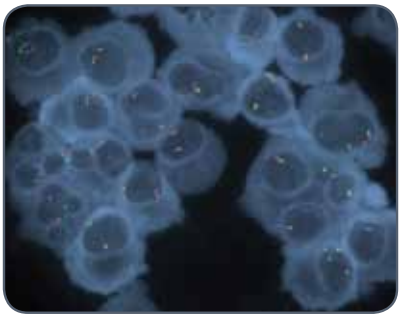
- Lipid antigen presentation to NKT cells[7]
- Intracellular calcium flux assays[8]
- Hybridoma generation[9]
- Adoptive transfer[10]



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:

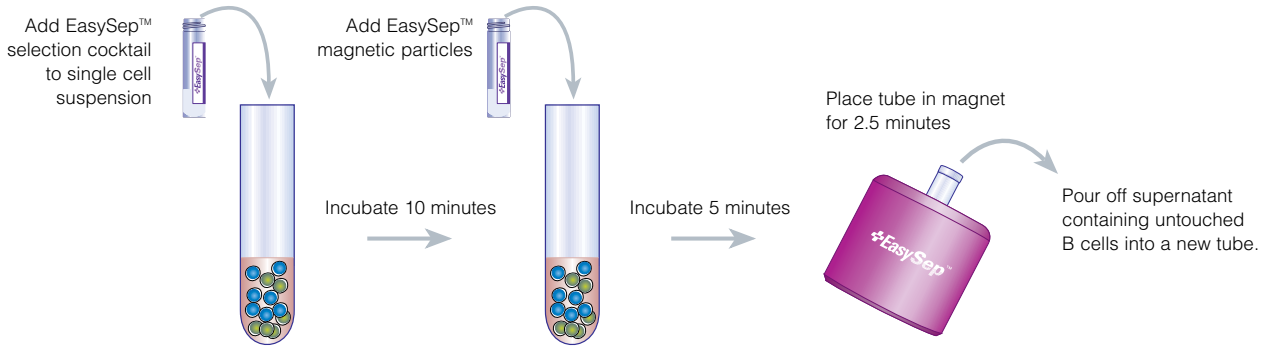
- Multiple Myeloma
- Chronic Lymphocytic Leukemia

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



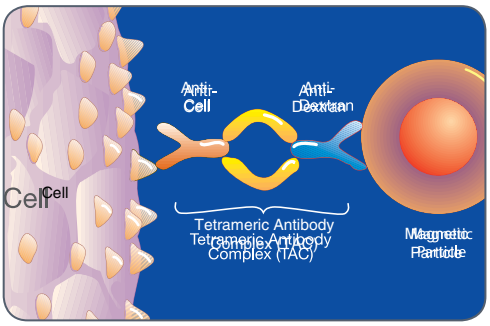
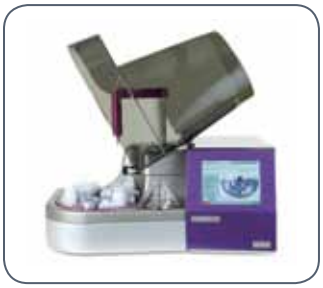


FIGURE 1. Tetrameric Antibody Complex (TAC) crosslinking a cell to a dextran coated magnetic particle.

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.

RosetteSep™ Human B Cell Enrichment Procedure

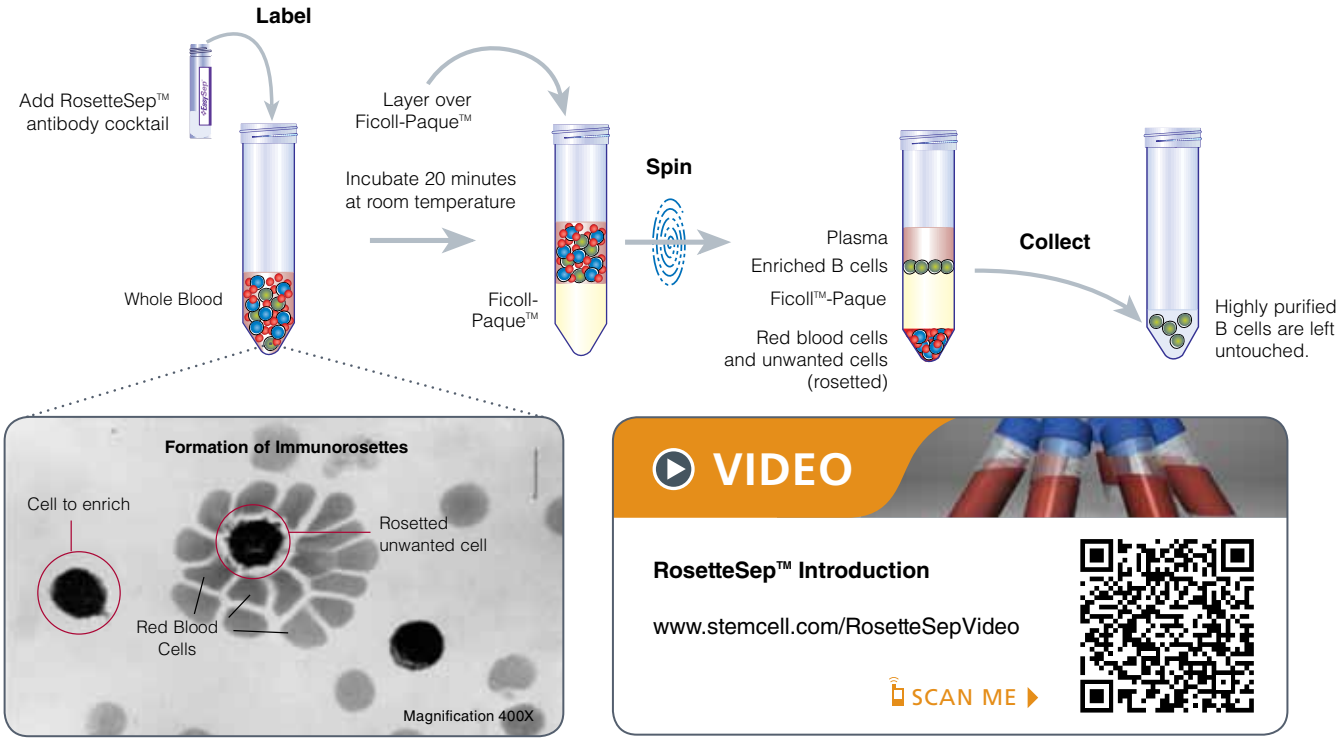


FIGURE 2. Image of a blood sample after addition of the RosetteSep™ cocktail, and prior to centrifugation over Ficoll™.

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 10^7 cells/mL, and red blood cells (RBCs) should be present at a ratio of at least 30 - 50 RBCs per nucleated cell.

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)

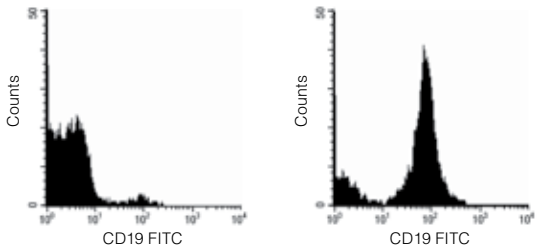
Isolate untouched human B cells directly from whole blood without magnets or special equipment.

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[8]
- Immunofluorescence and FISH analysis[11]
- Chromatin immunoprecipitation analysis[11]

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

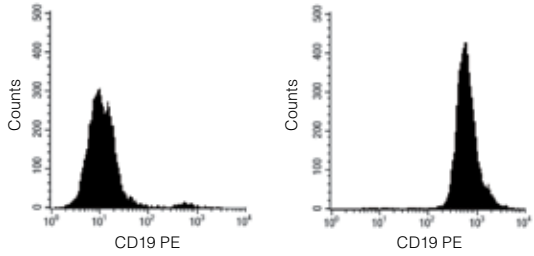
Isolate CD19$^+$ human B cells directly from whole blood.

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[9]
- Therapeutic monoclonal antibody production[9]
- Hybridoma optimization using morphogenics[9]

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)

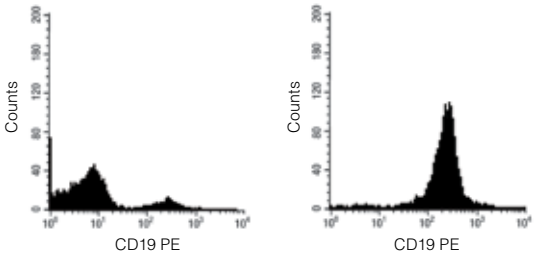
Isolate untouched B cells from PBMC or leukapheresis samples.

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

Start: 8% CD19⁺ cells | Enriched: 99% CD19⁺ cells



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[7]
- DNAse protection assays[12]
- Gene expression profiling by microarray analysis[13]

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

CLL Research Compatible

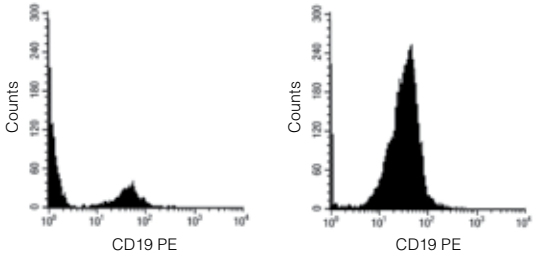
Isolate untouched human B cells from PBMC samples that include B cells that express CD43 (e.g. CLL samples).

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)

Start: 11% CD19⁺ cells | Enriched: 96% CD19⁺ cells



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)[14]
- Analyzing the specificity of cytotoxic drugs for CLL[15]
- Gene expression profiling in CLL[16]

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.

Human Naïve and Memory B Cells

Starting with PBMC or Leukapheresis Samples

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.

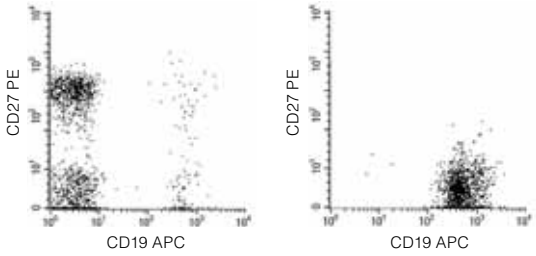
HOW IT WORKS:

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 Naive 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.

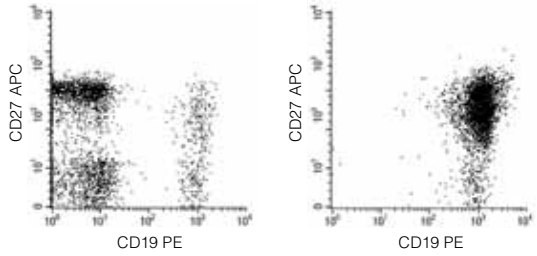
HOW IT WORKS:

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)

For Multiple Myeloma Research

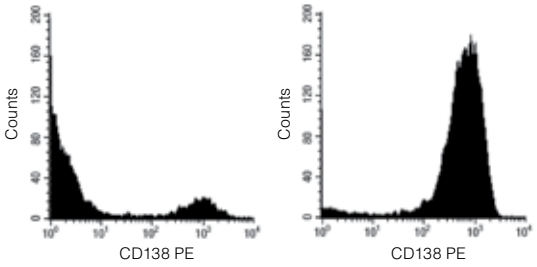
Isolate CD138+ plasma cells from PBMC or bone marrow.

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

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)

For Multiple Myeloma Research

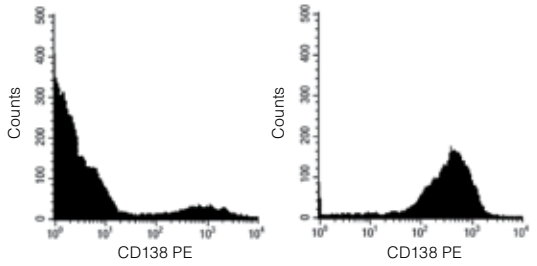
Isolate CD138+ plasma cells from whole blood.

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

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 myeloma[17], primary systemic amyloidosis[18] and plasma cell leukemia[19].

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.

Mouse B Cells

Starting With Spleen Or Other Tissues

Isolate untouched mouse B cells by negative selection, or positively select CD19⁺ B cells directly from single cell suspensions of spleen or other tissues.

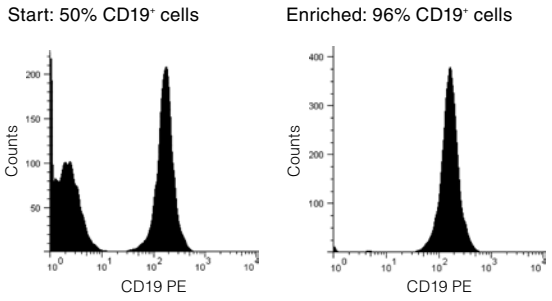
EasySep™ Mouse B Cell Enrichment Kit (Catalog #19754)

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



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[22]

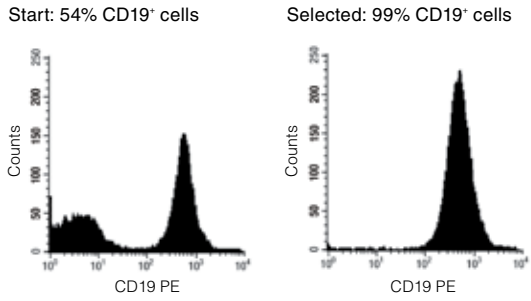
EasySep™ Mouse CD19 Positive Selection Kit (Catalog #18754)

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



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

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

Antibodies

PRODUCTS	DESCRIPTION	CATALOG #
CD19 Antibody, Clone 4G7, PE-Conjugated	Mouse Monoclonal Antibody to Human CD19 - PE Conjugated	10509
CD19 Antibody, Clone 4G7, FITC-Conjugated	Mouse Monoclonal Antibody to Human CD19 - FITC Conjugated	10409
CD20 Antibody, Clone L27, PE-Conjugated	Mouse Monoclonal Antibody to Human CD20 - PE Conjugated	10510
CD20 Antibody, Clone L27, FITC-Conjugated	Mouse Monoclonal Antibody to Human CD20 - FITC Conjugated	10410

Product Listing

Complete Product Listing

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

B Cell Kits for HLA Analysis

STARTING SAMPLE	B CELL PHENOTYPE	PRODUCT	CATALOG #
Whole blood	All B cells	RosetteSep™ HLA B Cell Enrichment Cocktail	15064HLA
		EasySep™/RoboSep™ HLA B Cell Enrichment: Complete Processing Kit for Whole Blood	19954HLA/19954HLARF
	CD19+/CD20+	EasySep™/RoboSep™ HLA Whole Blood B Cell Positive Selection Kit	18184HLA/18184HLARF
	CD19+	EasySep™/RoboSep™ Human Whole Blood CD19 Positive Selection Kit	18084/18084RF
	CD20+	EasySep™/RoboSep™ Human Whole Blood CD20 Positive Selection Kit	18685/18685RF
PBMC	All B cells	EasySep™/RoboSep™ HLA B Cell Enrichment Kit	19054HLA/19054HLARF
	CD19+/CD20+	EasySep™/RoboSep™ HLA B Cell Positive Selection Kit	18454HLA/18454HLARF
	CD19+	EasySep™/RoboSep™ Human CD19 Positive Selection Kit	18054/18054RF

References

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3. Darce J, Arendt B, Chang SK et al. Divergent Effects of BAFF on human memory B cell differentiation into Ig-secreting cells. J. Immunol. 178:5612-5622, 2007
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10. Gigley J, Fox B, and Bzik D. Cell-mediated immunity to toxoplasma gondii develops primarily by local Th1 host immune responses in the absence of parasite replication. J. Immunol. 182:1069–1078, 2009
11. Brugat T, Nguyen-Khac F, Grelier A et al. Telomere dysfunction-induced foci arise with the onset of telomeric deletions and complex chromosomal aberrations in resistant chronic lymphocytic leukemia cells. Blood 116:239-249, 2010
12. Mihalcik S, Huddleston P, Wu X et al. The structure of the TNFRSF13C promoter enables differential expression of BAFF-R during B cell ontogeny and terminal differentiation. J. Immunol. 185:1045–1054, 2010
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