

# EasySep™ Human B Cell Isolation Kit

For processing 1 x 10<sup>9</sup> cells

Catalog #17954  
Catalog #17954RF RoboSep™

Negative Selection

Document #1000005312 | Version 03



Scientists Helping Scientists™ | [WWW.STEMCELL.COM](http://WWW.STEMCELL.COM)

TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713

INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM

FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

## Description

Isolate untouched and highly purified B cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or washed leukapheresis samples in as little as 9 minutes by immunomagnetic negative selection.

- Fast, easy-to-use, and column-free
- Up to 96% purity with high recovery
- Isolated cells are untouched

This kit targets non-B cells for removal with antibodies recognizing specific surface markers. Unwanted cells are labeled with antibodies and magnetic particles and separated without columns using an EasySep™ magnet. Desired cells are simply poured off into a new tube. Isolated cells are immediately available for downstream applications, such as flow cytometry, culture, or DNA/RNA extraction.

## Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human B Cell Isolation Cocktail	17954C	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.
EasySep™ Isolation Cocktail Enhancer	17900	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A solution that enhances the performance of the isolation cocktail.
EasySep™ Dextran RapidSpheres™ 50102	50102	1 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles in water.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

## Sample Preparation

For available fresh samples, see [www.stemcell.com/primarycells](http://www.stemcell.com/primarycells).

### PERIPHERAL BLOOD

Prepare a PBMC suspension from whole blood (e.g. Human Whole Peripheral Blood\*, Catalog #70507) by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07811). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD\*\* (Catalog #85450/85415) cell isolation tube, or source fresh PBMCs (e.g. Human Peripheral Blood Mononuclear Cells, Fresh\*, Catalog #200-0077).

If using previously frozen PBMCs (e.g. Human Peripheral Blood Mononuclear Cells, Frozen\*, Catalog #70025), incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes. It is recommended to wash the cells at least twice with a medium or buffer of choice (e.g. DMEM, IMDM, RPMI, or PBS containing 10% fetal bovine serum [FBS]) prior to labeling and separation. Filter aggregated suspensions through a 37 µm cell strainer (Catalog #27250) for optimal results.

After preparation, resuspend cells at 5 x 10<sup>7</sup> cells/mL in recommended medium.

### LEUKAPHERESIS

Wash the peripheral blood leukapheresis sample (e.g. Human Peripheral Blood Leukopak, Fresh\*, Catalog #70500) by adding an equivalent volume of recommended medium or PBS containing 2% FBS. Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). If red blood cell lysis is desired, lyse with Ammonium Chloride Solution (Catalog #07800). If platelet removal is desired, centrifuge at 120 x g for 10 minutes with the brake off. Carefully remove the supernatant and resuspend the cells at 5 x 10<sup>7</sup> cells/mL in recommended medium..

\* Some primary cell products are available only in select regions. Contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com) for further information.

\*\* SepMate™ IVD is available only in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. In all other regions, SepMate™ is available for research use only (RUO).



## Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of Ca++ and Mg++.

## Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.




**Table 1. EasySep™ Human B Cell Isolation Kit Protocol**

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 <b>EasySep™</b> (Catalog #18000)	<b>“The Big Easy”</b> (Catalog #18001) 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10 <sup>7</sup> cells/mL 0.25 - 2 mL	5 x 10 <sup>7</sup> cells/mL 0.5 - 8 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add Cocktail Enhancer to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
3	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
5	Add RapidSpheres™ to sample and mix.	50 µL/mL of sample NO INCUBATION, proceed immediately to next step	50 µL/mL of sample NO INCUBATION, proceed immediately to next step
6	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 2.5 mL	<ul style="list-style-type: none"> <li>• Top up to 5 mL for samples ≤ 4 mL</li> <li>• Top up to 10 mL for samples &gt; 4 mL</li> </ul>
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 minutes
7	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
8	Remove the tube from the magnet; place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 1 minute	RT for 1 minute
9	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

\* Leave the magnet and tube inverted for 2 - 3 seconds, then return upright. Do not shake or blot off any drops that may remain hanging from the mouth of the tube.

Table 2. EasySep™ Human B Cell Isolation Kit Protocol

STEP	INSTRUCTIONS	EASYSEP™ MAGNETS		
		 <b>EasyEights™ (Catalog #18103)</b> <div>5 mL tube</div>	 <div>14 mL tube</div>	 <b>Easy 50 (Catalog #18002)</b>
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10 <sup>7</sup> cells/mL 0.5 - 2 mL	5 x 10 <sup>7</sup> cells/mL 1 - 8.5 mL	5 x 10 <sup>7</sup> cells/mL 10 - 45 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add Cocktail Enhancer to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
3	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
5	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes	RT for 3 minutes
6	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 2.5 mL	<ul style="list-style-type: none"> <li>• Top up to 5 mL for samples ≤ 4 mL</li> <li>• Top up to 10 mL for samples &gt; 4 mL</li> </ul>	<ul style="list-style-type: none"> <li>• Top up to 25 mL for samples ≤ 20 mL</li> <li>• Top up to 50 mL for samples &gt; 20 mL</li> </ul>
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
7	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
8	Remove the tube from the magnet; place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
9	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Isolated cells are ready for use	Isolated cells are ready for use	Isolated cells are ready for use


RT - room temperature (15 - 25°C)

\*\* Collect the entire supernatant, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube, use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube, use a 10 mL serological pipette [Catalog #38004]).

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ Human B Cell Isolation Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000)	
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10 <sup>7</sup> cells/mL 0.5 - 8 mL	
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	
2	Select protocol.	Human B Cell Negative Isolation Kit - 17954	
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	
4	Load the carousel.	Follow on-screen prompts	
	Start the protocol.	Press the green "Run" button	
5	Unload the carousel when the run is complete.	Isolated cells are ready for use	

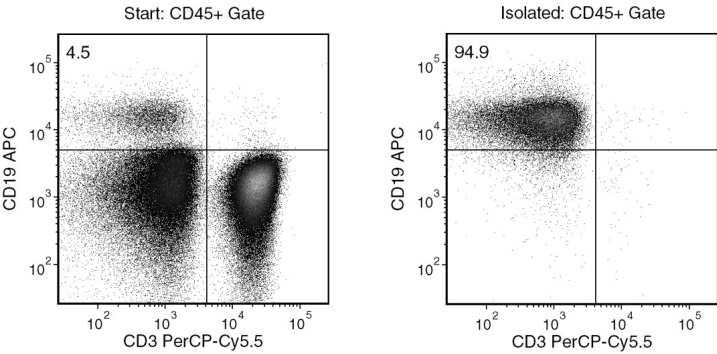
Notes and Tips

ASSESSING PURITY

For purity assessment of B cells (CD3-CD19+) by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005)
- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011; optional)
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018; optional)

Data



Starting with human PBMCs, the B cell content (CD3-CD19+) of the isolated fraction is typically 95.1 ± 1.4% (mean ± SD). In the above example, the purities of the start and final isolated fractions are 4.5% and 94.9%, respectively.

PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED. FOR ADDITIONAL INFORMATION ON QUALITY AT STEMCELL, REFER TO [WWW.STEMCELL.COM/COMPLIANCE](http://WWW.STEMCELL.COM/COMPLIANCE).

Copyright © 2025 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, EasySep, RapidSpheres, and RoboSep are trademarks of STEMCELL Technologies Canada Inc. Corning is a registered trademark of Corning Incorporated. All other trademarks are the property of their respective holders. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.