

EasySep™ Human B Cell Enrichment Kit

Negative Selection
Catalog #19054

For processing 1 x 10⁹ cells



Scientists Helping Scientists™ | 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

Document #28744 | Version 4_4_1

Description

Isolate untouched and highly purified B cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or lysed leukapheresis samples by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 99% purity
- Untouched, viable cells

This kit targets non-B cells for removal with antibodies recognizing specific cell 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 Enrichment Cocktail	19054C.2	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™ D Magnetic Particles	19250	2 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles in TBS.

PBS - phosphate-buffered saline; TBS - TRIS-buffered saline

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

Sample Preparation

For available fresh and frozen samples, see www.stemcell.com/primarycells.

PERIPHERAL BLOOD

Prepare a PBMC suspension from whole peripheral blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation without the need for careful sample layering, use the SepMate™-15 (Catalog #15415) or SepMate™-50 (Catalog #15450) cell isolation tube.

If using previously frozen PBMCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL for at least 15 minutes at room temperature (15 - 25°C) prior to labeling and separation. Filter clumpy suspensions through a 40 µm Cell Strainer (Catalog #27305) for optimal results.

After preparation resuspend cells at 5 x 10⁷ cells/mL in recommended medium.

LEUKAPHERESIS (LEUKO PAK)

If working with large volumes (> 150 mL), concentrate leukapheresis cells first by centrifuging at 500 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original leukapheresis volume with recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of recommended medium). For small volumes (≤ 150 mL), add Ammonium Chloride Solution (Catalog #07800) directly to the cell suspension.

1. Add an equal volume of Ammonium Chloride Solution to the leukapheresis.
2. Incubate for 15 minutes on ice.
3. Centrifuge at 500 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 150 x g for 10 minutes at room temperature with the brake off. Carefully remove the supernatant.
5. Repeat step 4 one or more times until most of the platelets have been removed (indicated by a clear supernatant).
6. Resuspend the cells at 5 x 10⁷ cells/mL in recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% fetal bovine serum (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 Enrichment Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 EasySep™ (Catalog #18000)	 “The Big Easy” (Catalog #18001)
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10 ⁷ cells/mL 0.5 - 2 mL	5 x 10 ⁷ cells/mL 0.5 - 8.5 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Add Enrichment Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
3	Vortex Magnetic Particles.	30 seconds	30 seconds
4	Add Magnetic Particles to sample.	75 µL/mL of sample	75 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	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"> • Top up to 5 mL for samples < 4 mL • Top up to 10 mL for samples ≥ 4 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
6	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off 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 Enrichment Kit Protocol

		EASYSEP™ MAGNETS			
STEP	INSTRUCTIONS	 EasyPlate™ (Catalog #18102)	EasyEights™ (Catalog #18103)		 Easy 50 (Catalog #18002)
			 5 mL tube	 14 mL tube	
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10 ⁷ cells/mL 0.05 - 0.2 mL	5 x 10 ⁷ cells/mL 0.25 - 2 mL	5 x 10 ⁷ cells/mL 0.5 - 8 mL	5 x 10 ⁷ cells/mL 1 - 40 mL
	Add sample to required tube (or plate when using the EasyPlate™ EasySep™ Magnet).	Round bottom, non-tissue culture-treated 96-well plate (e.g. Costar Catalog #3788 or Corning Catalog #351177)	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)	50 mL conical tube (e.g. Corning Catalog #352070)
2	Add Enrichment Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
3	Vortex Magnetic Particles.	30 seconds	30 seconds	30 seconds	30 seconds
4	Add Magnetic Particles to sample.	100 µL/mL of sample	75 µL/mL of sample	75 µL/mL of sample	75 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
5	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 0.25 mL	Top up to 2.5 mL	<ul style="list-style-type: none"> • Top up to 5 mL for samples < 4 mL • Top up to 10 mL for samples ≥ 4 mL 	<ul style="list-style-type: none"> • Top up to 25 mL for samples ≤ 10 mL • Top up to 50 mL for samples > 10 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
6	Carefully pipette** (do not pour) the enriched cell suspension into a new tube or plate.	Isolated cells are ready for use	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 the EasyEights™ 5 mL tube use a 2 mL serological pipette and for the EasyEights™ 14 mL tube use a 10 mL serological pipette)

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 Enrichment Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000)
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10 ⁷ cells/mL 0.5 - 8.5 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Select protocol.	Human B Cell Negative Selection 19054
3	Vortex Magnetic Particles.	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

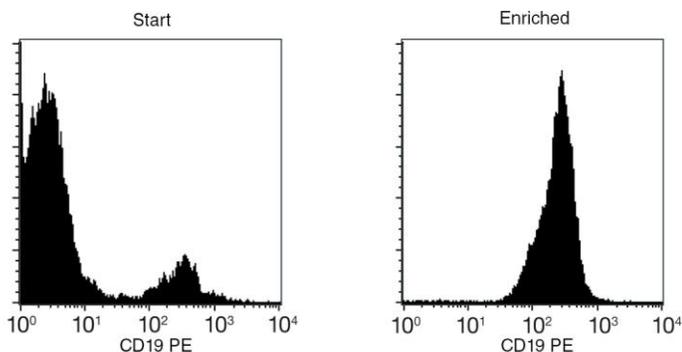
Notes and Tips

ASSESSING PURITY

For purity assessment of B cells by flow cytometry use fluorochrome-conjugated:

- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005), or
- Anti-Human CD20 Antibody, Clone 2H7 (Catalog #60008)

Data



Starting with mononuclear cells, the CD19+ cell content of the enriched fraction typically ranges from 95 - 99%. In the example above, the final purities of the start and enriched fractions are 8% and 99% respectively.

STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485. PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED.

Copyright © 2015 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, EasyEights, EasyPlate, EasySep, RoboSep, and SepMate are trademarks of STEMCELL Technologies Inc. Lymphoprep is a trademark of AXIS-SHIELD. 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.