



## EasySep™ Human B Cell Enrichment Kit without CD43 Depletion

Negative Selection  
Catalog #19154

For processing  $1 \times 10^9$  cells



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## Description

Isolate untouched and highly purified B cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) of patients with B cell leukemia or lymphoma, or with other disease states in which B cells may express CD43 by immunomagnetic negative selection.

- Fast and easy-to-use
- Up to 98% purity
- Isolated cells are untouched

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.

- For isolating CD43-negative B cells from normal samples, use EasySep™ Human B Cell Enrichment Kit (Catalog #19054).
- For isolating B cells including plasma cells from non-leukemia or lymphoma samples, use EasySep™ Human Pan-B Cell Enrichment Kit (Catalog #19554).

## Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human B Cell Enrichment Cocktail w/o CD43 Depletion	19154C	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™ Magnetic Nanoparticles	19150.1	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 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 and frozen samples, see [www.stemcell.com/primarycells](http://www.stemcell.com/primarycells).

### PERIPHERAL BLOOD

Prepare a PBMC suspension from whole blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD\* (Catalog #85450/85415) cell isolation tube.

If using previously frozen PBMCs, 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 prior to labeling and separation. Filter aggregated suspensions through a 40 µm Cell Strainer (Catalog #27305) for optimal results.

After preparation, resuspend cells at  $5 \times 10^7$  cells/mL in recommended medium.

\* SepMate™ IVD is only available 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).

### LEUKAPHERESIS (LEUKO PAK)

If working with large volumes (> 150 mL), concentrate leukapheresis sample 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 leukapheresis sample.

1. Add an equal volume of Ammonium Chloride Solution to the leukapheresis sample.
2. Incubate on ice for 15 minutes.
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  $5 \times 10^7$  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<sup>++</sup> and Mg<sup>++</sup>.

**Directions for Use – Manual EasySep™ Protocols**

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

**Table 1. EasySep™ Human B Cell Enrichment Kit without CD43 Depletion 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 - 8.5 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	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times	Pipette up and down more than 5 times
4	Add Magnetic Particles to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 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"> <li>• Top up to 5 mL for samples &lt; 2 mL</li> <li>• Top up to 10 mL for samples ≥ 2 mL</li> </ul>
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 10 minutes
6	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
OPTIONAL ADDITIONAL SEPARATION for PURITY NOTE: This will improve purity but may reduce recovery		---	---
7	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 10 minutes
8	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
OPTIONAL ADDITIONAL SEPARATION for RECOVERY NOTE: This will improve recovery but may reduce purity		---	---
7	Remove the tube from the magnet and add recommended medium to indicated volume. Mix by gently pipetting up and down 5 - 6 times.	Top up to 2.5 mL	Top up to 5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 10 minutes
8	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.	Combine with the poured-off fraction from step 6 Isolated cells are ready for use	Combine with the poured-off fraction from step 6 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.

## Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 2 for detailed instructions regarding the RoboSep™ procedure.

**Table 2. RoboSep™ Human B Cell Enrichment Kit without CD43 Depletion Protocol**

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000)
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10 <sup>7</sup> 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.	<ul style="list-style-type: none"> <li>Human B Cell Negative Selection without CD43 19154-high purity</li> <li>Human B Cell Negative Selection without CD43 19154-high recovery</li> </ul>
3	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times
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

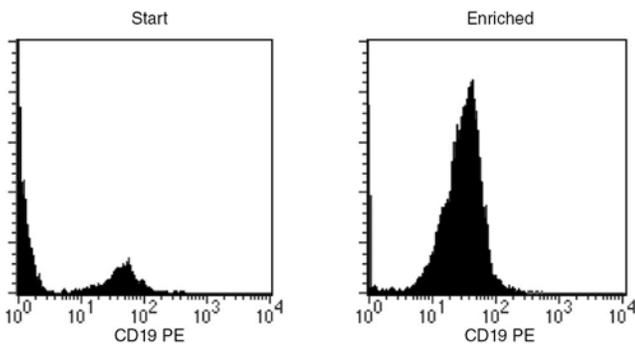
## Notes and Tips

### ASSESSING PURITY

For purity assessment by flow cytometry use one of the following fluorochrome-conjugated antibody clones:

- Anti-Human CD19 Antibody, Clone H1B19 (Catalog #60005), or
- Anti-Human CD20 Antibody, Clone 2H7 (Catalog #60008)

## Data



Starting with previously frozen PBMCs, the B cell content (CD19+) of the enriched fraction typically ranges from 87 - 98%. In the above example, the purities of the start and final enriched fractions are 11% and 96%, respectively.

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