EasySep™ Human Pan-B Cell Enrichment Kit

For processing 1 x 10^9 cells

Catalog #19554

#19554RF

Negative Selection

Document #10000000891 | Version 02

STEMCELL™ TECHNOLOGIES

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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 including plasma cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or lysed leukapheresis samples by immunomagnetic negative selection. This kit is not recommended for use with peripheral blood or other tissues of patients with B cell leukemia or lymphoma or with other disease states in which B cells may express CD36 and/or CD123.

- Fast, easy-to-use, and column-free
- Up to 99% 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 isolation of only CD43-negative B cells from normal samples, we recommend using EasySep™ Human B Cell Enrichment Kit (Catalog #19054).
- For enrichment of B cells from peripheral blood or other tissues of patients with B cell leukemia or lymphoma or with other disease states in which B cells may express CD43, CD36, and/or CD123, we recommend using EasySep™ Human B Cell Enrichment Kit II Without CD43 Depletion (Catalog #17963).

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human Pan-B Cell Enrichment Cocktail	19554C	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 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 37 µm cell strainer (Catalog #27215) for optimal results. After preparation, resuspend cells at 5 x 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).

LYSED LEUKAPHERESIS

1. Add 4 parts Ammonium Chloride Solution (Catalog #07800) to 1 part leukapheresis sample.
   NOTE: If working with large volumes (> 20 mL), concentrate the Leukopak first by centrifuging at 300 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original Leukopak volume with recommended medium (e.g. for 30 mL of cells, resuspend in 3 mL of recommended medium and add 12 mL of Ammonium Chloride Solution). For small volumes (≤ 20 mL), add Ammonium Chloride Solution directly to the Leukopak.
2. Incubate on ice for 15 minutes.
3. Wash the cells by topping up the tube with recommended medium. Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C) . Remove the supernatant.
4. OPTIONAL (FOR PLATELET REMOVAL):
   a. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 120 x g for 10 minutes at room temperature with the brake off. Carefully remove the supernatant.
   b. Repeat step 4a one or more times until most of the platelets have been removed (indicated by a clear supernatant).
5. Resuspend the cells at 5 x 10^7 cells/mL in recommended medium.

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 Pan-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. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Add Enrichment Cocktail to sample.** **NOTE: Do not vortex cocktail.**	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.** **NOTE: Particles should appear evenly dispersed.**	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	• Top up to 5 mL for samples < 2 mL • Top up to 10 mL for samples ≥ 2 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 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 Pan-B Cell Enrichment Kit Protocol

		EASYSEP™ MAGNET
STEP	INSTRUCTIONS	**Easy 50 (Catalog #18002)** 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 1 - 40 mL
	Add sample to required tube.	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add Enrichment Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes
3	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Add Magnetic Particles to sample.	75 µL/mL of sample
	Mix and incubate.	RT for 5 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 10 mL for samples < 5 mL • Top up to 20 mL for samples 5 - 10 mL • Top up to 30 mL for samples > 10 - 15 mL • Top up to 40 mL for samples > 15 - 20 mL • Top up to 50 mL for samples > 20 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes
6	Carefully pipette** (do not pour) off the enriched cell suspension into a new tube.	Isolated cells are ready for use

** Collect the entire supernatant, all at once, into a single 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 Pan-B Cell Enrichment Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #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 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Select protocol.	Human Pan-B Cell Negative Selection 19554
3	Vortex Magnetic Particles. 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 now ready for use

Notes and Tips

ASSESSING PURITY

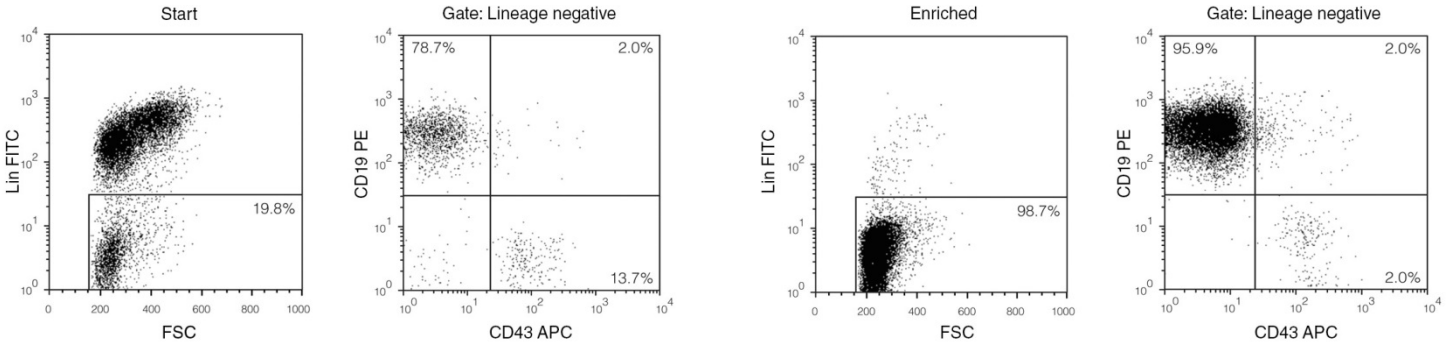
Pan-B cells are described here as lineage- (Lin; CD4, CD8, CD14, CD16, CD56) CD19+ and Lin-CD19-CD43+. For purity assessment of pan-B cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005), and
- Anti-Human CD43 Antibody, Clone CD43-10G7 (Catalog #60085), and
- Anti-human lineage-specific antibodies (see below)

For lineage-specific antigen labeling, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016), and
- Anti-Human CD8a Antibody, Clone RPA-T8 (Catalog #60022), and
- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004), and
- Anti-Human CD16 Antibody, Clone 3G8 (Catalog #60041), and
- Anti-Human CD56 Antibody, Clone HCD56 (Catalog #60021)

Data



Starting with nucleated cells, the pan-B cell content (Lin-CD19+ and Lin-CD19-CD43+) of the enriched fraction typically ranges from 90 - 99%. In the above example, the purities of the start and final enriched fractions are 18.7% and 98.6%, respectively.