EasySep™ Human Naïve B Cell Enrichment Kit

Negative Selection

Catalog #19254VX For processing 5 x 10^9 cells

STEMCELL™ TECHNOLOGIES
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

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Description

Isolate untouched and highly purified naïve B cells (CD19+CD27-) from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) by immunomagnetic negative selection.

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

This kit targets non-naïve 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 Naïve B Cell Enrichment Cocktail	19254CVX	1 x 5 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	19250VX	5 x 5 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 40 µm Cell Strainer (Catalog #27305) 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).

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 x 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++ 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 Naïve B Cell Enrichment Kit Protocol

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 0.25 - 1.75 mL	5 x 10^7 cells/mL 0.5 - 7.5 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 Selection 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. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add Magnetic Particles to sample.	250 µL/mL of sample	250 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
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 5 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 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
8	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 14 mL tube Combine with the poured-off fraction from step 6 Isolated cells are ready for use	• Use a new 14 mL tube for start samples < 2 mL • Use a new 50 mL tube for start samples ≥ 2 mL 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.

Table 2. EasySep™ Human Naïve 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 - 30 mL
	Add sample to required tube.	50 mL conical tube (e.g. Corning Catalog #352070)
2	Add Selection Cocktail to sample.	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.	250 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 10 minutes
6	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	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 10 minutes
8	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	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 2 - 3 times.	See step 5 for top up volumes
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes
8	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Use a new 50 mL tube Combine with the poured-off fraction from step 6 Isolated cells are ready for use

RT - room temperature (15 - 25°C)
** Collect the entire supernatant, all at once, into a single pipette.

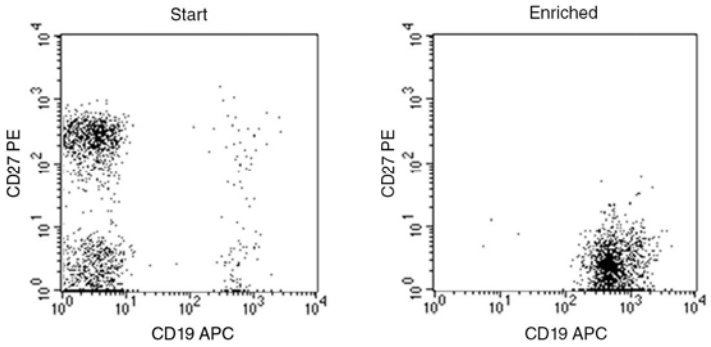
Notes and Tips

ASSESSING PURITY

For purity assessment of naïve B cells (CD19+CD27-) by flow cytometry use the following fluorochrome-conjugated antibodies:

- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005), and
- Anti-Human CD27 Antibody, Clone LG.3A10 (Catalog #60160)

Data



Starting with fresh mononuclear cells, the CD19+CD27- cell content of the enriched fraction is typically 92 - 98%. In the above example, the purities of the start and final enriched fractions are 4.3% and 97.5%, respectively.