



Negative Selection
Catalog #19056

EasySep™ Human Progenitor Cell Enrichment Kit

For processing 1×10^9 cells



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Description

Isolate untouched and highly purified hematopoietic progenitor cells from mobilized peripheral blood or from fresh or previously frozen bone marrow samples by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 70-fold enrichment
- Untouched, viable cells

This kit targets non-progenitor 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 enrichment of progenitor cells from cord blood and other cell preparations that contain large amounts of platelets, use EasySep™ Human Progenitor Cell Enrichment Kit with Platelet Depletion (Catalog #19356).

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human Progenitor Cell Enrichment Cocktail	19056C.1	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.

BONE MARROW and MOBILIZED PERIPHERAL BLOOD

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

Alternatively, remove red blood cells by lysis using Ammonium Chloride Solution (Catalog #07800).

If using previously frozen MNCs, 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 5×10^7 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 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% 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 Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human Progenitor 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 0.25 - 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 15 minutes	RT for 15 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 15 minutes	RT for 15 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 < 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 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.**	Use a new 5 mL tube	Use a new 14 mL tube
7	Remove the tube from the magnet (save tube if performing Optional Additional Separation) and place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 10 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.	Use a new 14 mL tube Isolated cells are ready for use	<ul style="list-style-type: none"> • Use a new 14 mL tube for start samples < 2 mL • Use a new 50 mL tube for start samples ≥ 2 mL Isolated cells are ready for use
OPTIONAL ADDITIONAL SEPARATION NOTE: This will improve recovery but may reduce purity		---	---
9	Remove the tube from the magnet in step 7 and add recommended medium to indicated volume. Mix by gently pipetting up and down 5 - 6 times.	Top up to 2.5 mL	<ul style="list-style-type: none"> • Top up to 5 mL for samples < 2 mL • Top up to 10 mL for samples ≥ 2 mL
10	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes
11	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension.	Combine with poured-off fraction from step 8 Isolated cells are ready for use	Combine with poured-off fraction from step 8 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.

** To improve recovery, a single round of magnetic separation may be completed by stopping the protocol after step 6. Purity may decrease by performing only a single round of separation.

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 Progenitor 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.	<ul style="list-style-type: none"> Human Progenitor Negative Selection 19056-high purity Human Progenitor Negative Selection 19056-high recovery 	
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

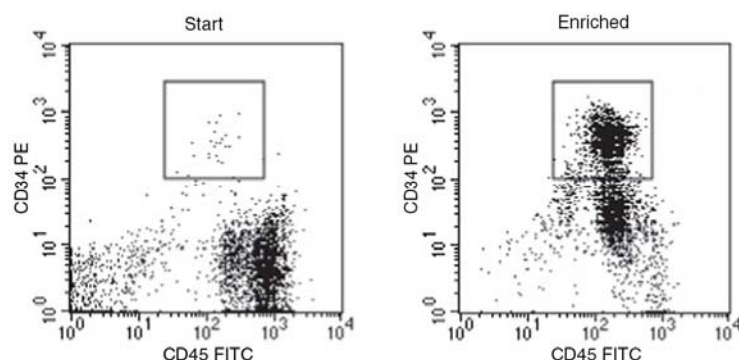
For purity assessment of hematopoietic progenitor cells by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013) or Clone 8G12 (Catalog #60121), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018; optional) or Clone 2D1 (Catalog #60123; optional)

Purity of CD34+ cells is typically expressed as a percentage of viable CD45+ cells. Viability is measured by exclusion of Propidium Iodide (Catalog #75002).

Purity of erythroid (BFU-E) and myeloid (CFU-GM) progenitor cells can be assessed in colony assays using semi-solid culture media such as MethoCult™ H4434 Classic (Catalog #04434) or MethoCult™ H4435 Enriched (Catalog #04435).

Data



The enrichment of CD34+ cells typically ranges between 15- and 70-fold relative to viable, CD45+ cells in unfractionated bone marrow (CD34+ cells: average fold enrichment \pm 1 SD is 42 ± 5 , n = 6). In the above example, the purities of the start and final enriched fractions are 0.7% and 47%, respectively.

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