EasySep™ Human Progenitor Cell Enrichment Kit II

For processing 1 x 10^9 cells

Catalog #17936

Negative Selection

Document #10000005221 | Version 01

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Description

Isolate untouched and highly purified hematopoietic progenitor cells from fresh human cord blood by immunomagnetic negative selection.

- Fast, easy-to-use, and column-free
- Up to 95% purity
- Isolated cells are untouched

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, DNA/RNA extraction, or adoptive transfer into mice.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human Progenitor Cell Enrichment Cocktail II	17936C	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™ Dextran RapidSpheres™ 50102	50102	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

WHOLE CORD BLOOD

Prepare a nucleated cell suspension from whole cord 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.

NOTE: For samples containing high red blood cell (RBC) contamination following density gradient centrifugation, RBCs may be removed by lysis using Ammonium Chloride Solution (Catalog #07800) prior to cell isolation.

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).

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++.

OR

RoboSep™ Buffer 2 (Catalog #20164), or PBS containing 0.5% bovine serum albumin (BSA) and 2 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 II 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 1 - 6 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.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
3	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
4	**Add RapidSpheres™ to sample.**	75 µL/mL of sample	75 µL/mL of sample
	Mix and incubate.	RT for 1 minute	RT for 1 minute
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 < 4 mL • Top up to 10 mL for samples ≥ 4 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 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 Isolated cells are ready for use	Use a new 14 mL tube Isolated cells are ready for use
OPTIONAL ADDITIONAL SEPARATION **NOTE: This will improve purity but may reduce recovery**		---	---
7	**Remove the tube from the magnet and place the new tube from step 6 (without lid) into the magnet and incubate for a second round of separation.**	RT for 1 minute	RT for 1 minute
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 5 mL tube Isolated cells are ready for use	Use a new 14 mL tube 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 Progenitor Cell Enrichment Kit II Protocol

STEP	INSTRUCTIONS	RoboSep™-S (Catalog #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 1 - 6 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Select protocol.	Human Progenitor Cell Enrichment 17936 - high purity OR Human Progenitor Cell Enrichment 17936 - high recovery
3	Vortex RapidSpheres™. 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 ready for use

Notes and Tips

ASSESSING PURITY

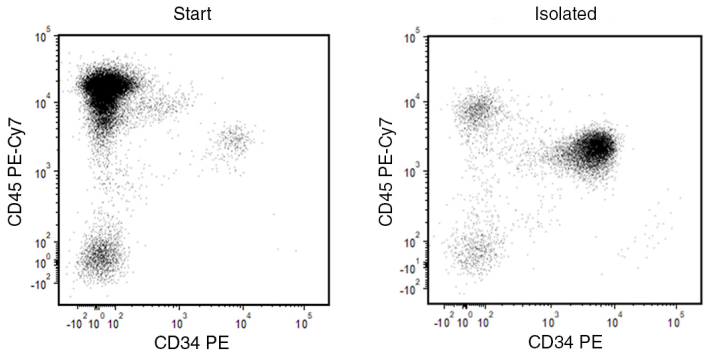
For purity assessment of CD34+ cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

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

Purity of CD34+ cells is typically expressed as a percentage of viable CD45+ cells. Viability is measured by exclusion of Propidium Iodide (Catalog #75002) or 7-AAD (7-Aminoactinomycin D; Catalog #75001).

Enriched CD34+ cells can be expanded and/or differentiated into mature hematopoietic cells of specific lineages using StemSpan™ serum-free expansion media and supplements (for more information, visit www.stemcell.com).

Data



Starting with 36- to 48-hour-old cord blood, the CD34+ cell content of the isolated fraction is typically 77.5 ± 16.0% (mean ± SD using the silver EasySep™ Magnet). In the above example, using fresh cord blood, the purities of the start and final isolated fractions are 0.7% and 82.4%, respectively.