EasySep™ Human Progenitor Cell Enrichment Kit with Platelet Depletion

For processing 1 x 10^9 cells

Catalog #19356
#19356RF RoboSep™

Negative Selection

Document #10000000888 | Version 01

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Description

Isolate untouched and highly purified hematopoietic progenitor cells from fresh or previously frozen human cord blood or other cell preparations that contain large numbers of platelets by immunomagnetic negative selection.

- Fast, easy-to-use, and column-free
- Up to 75% purity
- Isolated cells are untouched

This kit targets non-progenitor cells and platelets 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 Progenitor Cell Enrichment Cocktail with Platelet Depletion	19356C	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. Includes an Fc receptor blocking antibody.
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.

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.

If using previously frozen mononuclear cells, 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 clumpy suspensions through a 37 µm Cell Strainer (Catalog #27215) for optimal results.

Alternatively, red blood cells (RBCs) may be removed by lysis using Ammonium Chloride Solution (Catalog #07800).

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

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 with Platelet Depletion 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 2 - 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.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
3	**Vortex Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
4	**Add Magnetic Particles to sample.**	100 µL/mL of sample	100 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 10 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.**	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.

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 with Platelet Depletion 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 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Select protocol.	Human Progenitor Negative Selection with Platelet Depletion 19356
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 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).

The frequency of erythroid (BFU-E), myeloid (CFU-GM), and multilineage (CFU-GEMM) progenitor cells can be assessed in colony-forming unit assays in semi-solid culture media using MethoCult™ H4034 Optimum (Catalog #04034).

Data

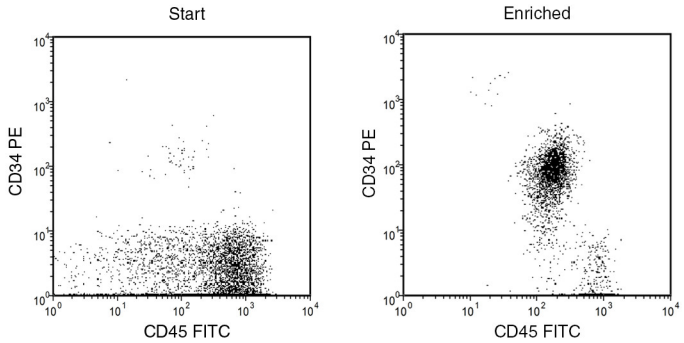

Figure 1. Typical EasySep™ Human Progenitor Cell Enrichment Kit with Platelet Depletion Profile

Starting with nucleated cells, the CD34+ cell content of the enriched fraction typically ranges from 50 - 75%, depending on the quality of the start sample. In the above example, the purities of the start and final enriched fractions are 2% and 87%, respectively.

NOTE: CD34+ enrichment is dependent on the frequency of CD34+ cells in the start sample, which is variable between cord blood samples. Use of poor quality cord blood or frozen samples may result in lower CD34+ cell content of the enriched fraction.