

# EasySep™ Human Progenitor Cell Enrichment Kit II

For processing 1 x 10<sup>9</sup> cells

Catalog #17936  
 Catalog #17936RF RoboSep™  
 Negative Selection



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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	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).

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<sup>7</sup> 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<sup>++</sup> and Mg<sup>++</sup>.

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<sup>++</sup> and Mg<sup>++</sup>.

## 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 <sup>7</sup> cells/mL 0.5 - 2 mL	5 x 10 <sup>7</sup> 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. 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 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	<ul style="list-style-type: none"> <li>• Top up to 5 mL for samples &lt; 4 mL</li> <li>• Top up to 10 mL for samples ≥ 4 mL</li> </ul>
	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; place the new tube from step 6 (without lid) into the magnet and incubate for a second 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 <sup>7</sup> 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

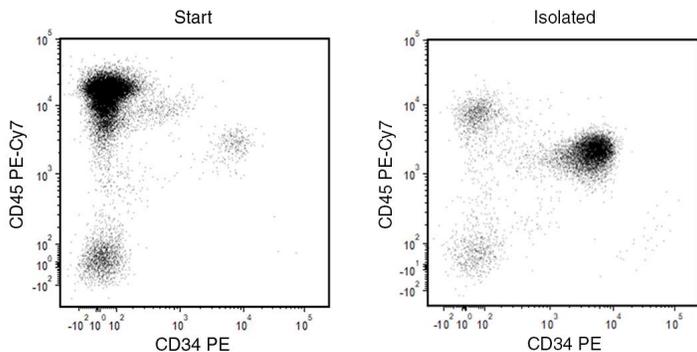
For purity assessment of CD34+ cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- 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](http://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 Big Easy" 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.

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