EasySep™ Human CD34 Positive Selection Kit II

For processing 1 x 10^10 cells using the Easy 250 EasySep™ Magnet

Catalog #100-1569

Positive Selection

Document #10000027274 | Version 02

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

Description

Isolate highly purified CD34+ cells from fresh mobilized leukapheresis samples by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 97% purity with high recovery
- No columns required

This kit targets CD34+ cells from mobilized leukapheresis samples for positive selection with an antibody recognizing the CD34 surface marker. Desired cells are labeled with antibodies and magnetic particles and separated without columns using an EasySep™ magnet. Unwanted cells are simply pipetted off, while desired cells remain in the flask. Isolated cells are immediately available for downstream applications, such as flow cytometry, culture, or DNA/RNA extraction.

NOTE: This is the Product Information Sheet (PIS) for isolating CD34+ cells using the Easy 250 EasySep™ Magnet (Catalog #100-0821). If using other magnets, refer to the applicable PIS, available at www.stemcell.com, or contact us to request a copy.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human CD34 Positive Selection Cocktail II	300-1044	1 x 10 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™ Dextran RapidSpheres™ 50103	50103	1 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, see www.stemcell.com/primarycells.

NOTE: Working with fresh lysed mobilized leukapheresis samples is recommended for optimal performance. Alternatively, washed mobilized leukapheresis samples may be used (see below) for faster sample processing, but a reduction in performance may be observed.

LYSED MOBILIZED LEUKAPHERESIS

1. Add an equal volume of Ammonium Chloride Solution (Catalog #07800) to the Leukopak (e.g. Human Mobilized Peripheral Blood Leukopak, Fresh, Catalog #200-0604).
   NOTE: If working with large volumes (> 150 mL), concentrate the Leukopak first by centrifuging at 300 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original Leukopak volume with the recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of the recommended medium and add 30 mL of Ammonium Chloride Solution). For small volumes (≤ 150 mL), add Ammonium Chloride Solution directly to the Leukopak.
2. Incubate on ice for 15 minutes.
3. Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. Wash the cells by topping up the tube with the recommended medium. Centrifuge the cells at 120 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 1 - 2 x 10^8 cells/mL in the recommended medium.

* Some primary cell products are available only in select regions. Contact us at techsupport@stemcell.com for further information.

WASHED LEUKAPHERESIS

Wash the fresh mobilized peripheral blood leukapheresis sample (e.g. Human Mobilized Peripheral Blood Leukopak, Fresh) by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS). Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). If platelet removal is necessary, centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at 1 - 2 x 10^8 cells/mL in the recommended medium.

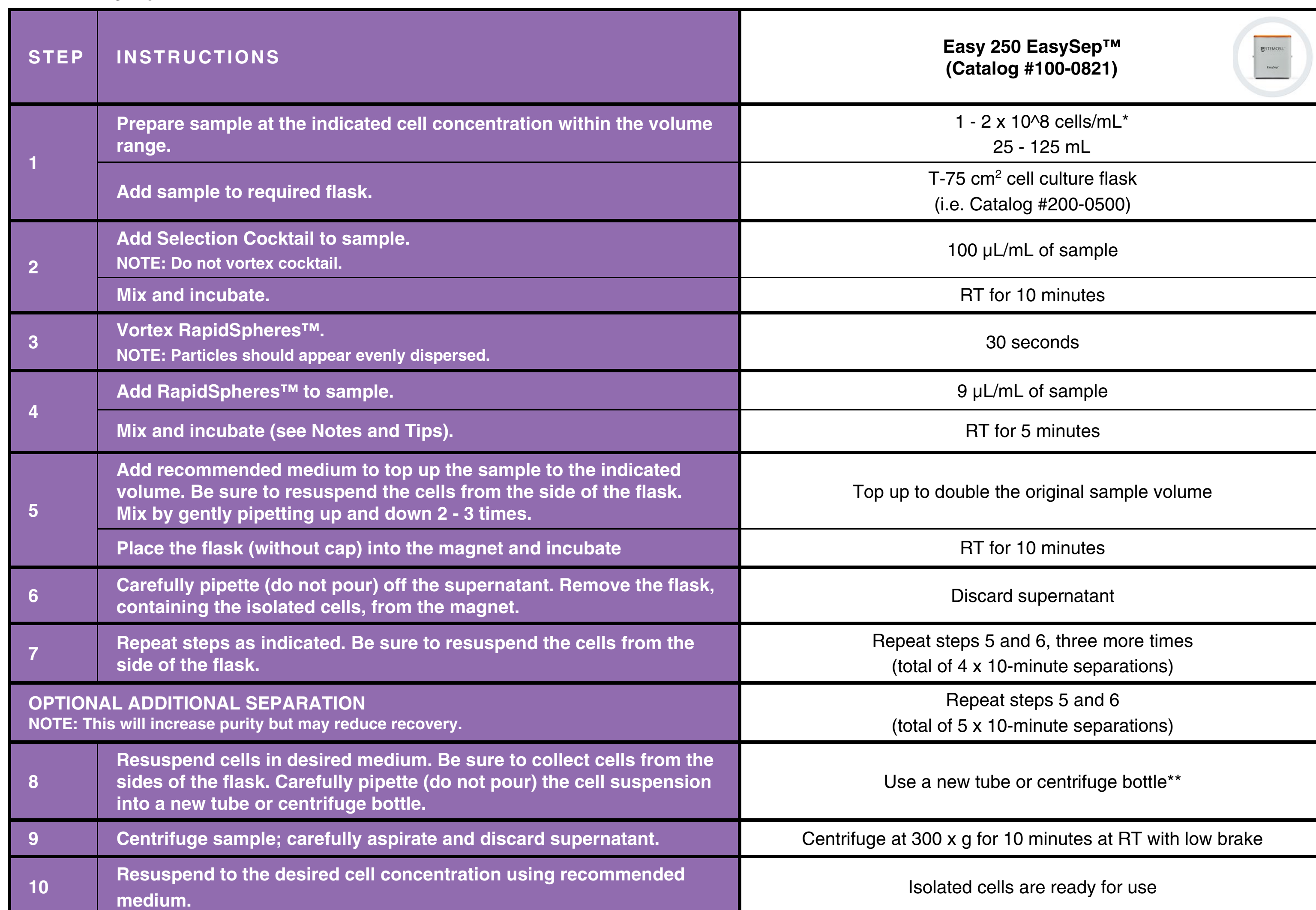
Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of Ca++ and Mg++.

Directions for Use – Manual EasySep™ Protocols

See pages 1 and 2 for Sample Preparation and Recommended Medium. Refer to Table 1 for detailed instructions regarding the EasySep™ procedure.

Table 1. EasySep™ Human CD34 Positive Selection Kit II Protocol



STEP	INSTRUCTIONS	Easy 250 EasySep™ (Catalog #100-0821)
1	Prepare sample at the indicated cell concentration within the volume range.	1 - 2 x 10^8 cells/mL* 25 - 125 mL
	Add sample to required flask.	T-75 cm^2 cell culture flask (i.e. Catalog #200-0500)
2	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample
	Mix and incubate.	RT for 10 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Add RapidSpheres™ to sample.	9 µL/mL of sample
	Mix and incubate (see Notes and Tips).	RT for 5 minutes
5	Add recommended medium to top up the sample to the indicated volume. Be sure to resuspend the cells from the side of the flask. Mix by gently pipetting up and down 2 - 3 times.	Top up to double the original sample volume
	Place the flask (without cap) into the magnet and incubate	RT for 10 minutes
6	Carefully pipette (do not pour) off the supernatant. Remove the flask, containing the isolated cells, from the magnet.	Discard supernatant
7	Repeat steps as indicated. Be sure to resuspend the cells from the side of the flask.	Repeat steps 5 and 6, three more times (total of 4 x 10-minute separations)
OPTIONAL ADDITIONAL SEPARATION NOTE: This will increase purity but may reduce recovery.		Repeat steps 5 and 6 (total of 5 x 10-minute separations)
8	Resuspend cells in desired medium. Be sure to collect cells from the sides of the flask. Carefully pipette (do not pour) the cell suspension into a new tube or centrifuge bottle.	Use a new tube or centrifuge bottle**
9	Centrifuge sample; carefully aspirate and discard supernatant.	Centrifuge at 300 x g for 10 minutes at RT with low brake
10	Resuspend to the desired cell concentration using recommended medium.	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
* Using 2 x 10^8 cell/mL may increase the purity but reduce recovery.
** e.g. 50 mL (30 x 115 mm) conical tube (Catalog #38010) or 225 mL centrifuge bottle (Corning Catalog #352075)

Notes and Tips

- After the addition of the Cocktail and RapidSpheres™, mix the sample with a 25 mL or 50 mL serological pipette (e.g. Catalog #38005/38006). Mixing can also be performed by rotating or gently agitating the flask. Cap the flask first to prevent spillage.
- To collect the supernatant, gently sweep the pipette back and forth along the midline of the T-75 cm^2 flask while aspirating. Avoid touching the sides of the flask. Switch to a 10 mL or smaller serological pipette to collect the residual supernatant. Removal of the residual supernatant is required to obtain high purity.
- To reduce debris in the isolated fraction, wash the cells in desired medium and centrifuge at 120 x g for 10 minutes at room temperature with the brake off. Carefully remove the supernatant then resuspend in desired medium.
- Depending on the quality and age of the Leukopak, the cells may aggregate during or before the cell separation protocol. To obtain optimal purity and recovery, it may be necessary to pass the cells through a 70 µm strainer to remove aggregates:
  - If the cells start to aggregate after sample preparation but prior to the cell separation protocol, strain the cells by passing them through a pre-wetted 70 µm strainer into the T-75 cm^2 flask before proceeding.
  - If the cells start to aggregate during the cell separation protocol, after the second magnetic separation (step 7), top up the flask to the recommended volume of buffer and resuspend well with a 25 mL pipette, focusing on removing the cells from the walls of the flask and breaking up the aggregates. Pass the contents of the flask through a pre-wetted 70 µm strainer into a new flask and continue with the third magnetic separation.

ASSESSING PURITY

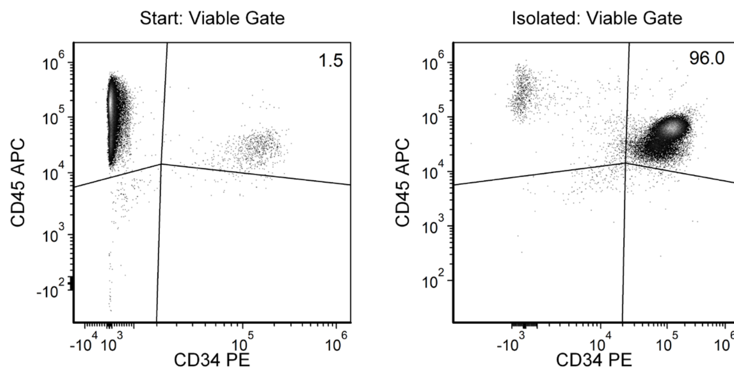
EasySep™ Human CD34 Positive Selection Cocktail II uses a class II anti-CD34 antibody clone that may block some class I and II anti-CD34 antibody clones used to assess purity by flow cytometry. For purity assessment of CD34+ cells by flow cytometry, use one of the following class III fluorochrome-conjugated antibody clones:

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

NOTE: Include a viability dye if necessary (e.g. Propidium Iodide [Catalog #75002] or 7-AAD [7- Aminoactinomycin D; Catalog #75001]).

Flow cytometry analysis of the positively selected cells may show slightly increased side scatter relative to the start sample.

Data



Starting with a mobilized Leukopak sample, the CD34+ cell content of the isolated fraction is typically 94.6 ± 2.4% (mean ± SD using the Easy 250 EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 1.5% and 96.0%, respectively.

PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED. FOR ADDITIONAL INFORMATION ON QUALITY AT STEMCELL, REFER TO WWW.STEMCELL.COM/COMPLIANCE.

Copyright © 2025 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, EasySep, and RapidSpheres are trademarks of STEMCELL Technologies Inc. Corning is a registered trademark of Corning Incorporated. All other trademarks are the property of their respective holders. All other trademarks are the property of their respective holders. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.