



EasySep™ Human Biotin Positive Selection Kit

Positive Selection
Catalog #18553

For processing 1 x 10⁹ cells



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

Document #28513 | Version 4_2_1

Description

Isolate highly purified cells labeled with biotinylated antibodies from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or other single-cell suspensions by immunomagnetic positive selection.

- Fast and easy-to-use
- No columns required

This kit targets cells labeled with biotinylated antibodies (not provided) for positive selection with an antibody recognizing biotin. Desired cells are labeled with antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Unwanted cells are simply poured off, while desired cells remain in the tube. Isolated cells are immediately available for downstream applications such as flow cytometry, cell culture, or cell-based assays.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Biotin Selection Cocktail	18153	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 Positive Selection	18150	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.
Anti-Human CD32 (Fc gamma RII) Blocker, for positive selection	18520	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.
RoboSep™ Vial	18550	1 vial	Not applicable	Not applicable	Not applicable

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.

PERIPHERAL BLOOD

Prepare a PBMC suspension from whole 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 PBMCs, 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 1 x 10⁸ 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⁺⁺.

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 Biotin Positive Selection 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.	1 x 10 ⁸ cells/mL 0.1 - 2.5 mL NOTE: If starting with fewer than 1 x 10 ⁷ cells, resuspend cells in 0.1 mL. For samples with a starting frequency of desired cells < 2%, start with a concentration of 2 x 10 ⁸ cells/mL.	1 x 10 ⁸ cells/mL 0.25 - 8 mL NOTE: If starting with fewer than 2.5 x 10 ⁷ cells, resuspend cells in 0.25 mL. For samples with a starting frequency of desired cells < 2%, start with a concentration of 2 x 10 ⁸ cells/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 FcR blocker to sample and mix.	100 µL/mL of sample	100 µL/mL of sample
3	Add biotinylated antibody to sample.‡	0.3 - 3.0 µg/mL of sample	0.3 - 3.0 µg/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
OPTIONAL WASH STEP may improve performance. Add recommended medium to top up the sample to the indicated volume and centrifuge. Resuspend sample in original volume.		Top up with 10-fold excess recommended medium and centrifuge. Carefully aspirate and discard supernatant. Resuspend in the same volume as step 1.	Top up with 10-fold excess recommended medium and centrifuge. Carefully aspirate and discard supernatant. Resuspend in the same volume as step 1.
4	Add Selection Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
5	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
6	Add Magnetic Particles to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
7	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 < 1 mL • Top up to 10 mL for samples ≥ 1 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes*	RT for 5 minutes*
8	Pick up the magnet, and in one continuous motion invert the magnet and tube,** pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
9	Repeat steps as indicated.	Steps 7 and 8, two more times (total of 3 x 5-minute separations)	Steps 7 and 8, two more times (total of 3 x 5-minute separations)
Continue on to next page.		Continue on to next page.	Continue on to next page.

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS (CONTINUED)	 EasySep™ (Catalog #18000)	"The Big Easy" (Catalog #18001) 
OPTIONAL ADDITIONAL SEPARATION(S) For samples with a starting frequency of desired cells < 15% NOTE: This will improve purity but may reduce recovery		Repeat steps 7 and 8, up to three more times (total of 4 - 6 x 5-minute separations)	Repeat steps 7 and 8, up to three more times (total of 4 - 6 x 5-minute separations)
10	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

‡ Titrate biotinylated antibody for optimal purity and recovery.

* Recovery may be improved by increasing separation time in the magnet to 10 minutes for each round.

** 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 Biotin Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000)
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10 ⁸ cells/mL 0.25 - 8 mL NOTE: If starting with fewer than 2.5 x 10 ⁷ cells, resuspend cells in 0.25 mL. For samples with a starting frequency of desired cells < 2%, start with a concentration of 2 x 10 ⁸ cells/mL.
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Add FcR blocker to sample and mix.	100 µL/mL of sample
3	Select protocol.	Human Biotin Positive Selection 18553-high purity
4	Transfer biotinylated antibody to the RoboSep™ Vial provided.	Use of this vial is required for RoboSep™ to run properly
5	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times
6	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green "Run" button
7	Unload the carousel when the run is complete. Remove the tube containing the isolated cells and resuspend in desired medium. Be sure to collect cells from the sides of the tube.	Isolated cells are ready for use

Notes and Tips

OPTIMIZING PURITY

Purity can be increased, for some cell types, by decreasing the amount of EasySep™ Biotin Selection Cocktail added. This may decrease recovery but will also reduce side scatter during subsequent flow cytometry analysis.

OPTIMIZING RECOVERY

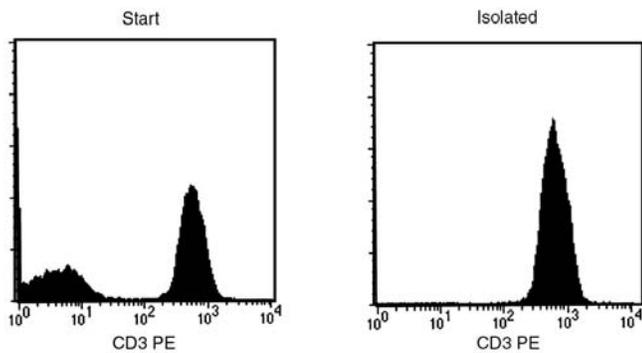
Recovery of positively selected biotin-labeled cells is dependent on the quality of the biotinylated antibody used. Antibodies that have expired or that have been stored improperly may show lower affinity for the surface marker on the target cell, resulting in lower recovery.

ASSESSING PURITY

For purity assessment of biotinylated cells by flow cytometry use one of the following methods:

- Add fluorochrome-conjugated antibody to the selected cells.
NOTE: The biotinylated antibody may block the labeling antibody.
- Use fluorochrome-conjugated antibodies to alternative cell surface markers.
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

Data



Starting with human PBMCs, the purities of the start and final isolated fractions, assessed using a PE-conjugated anti-human CD3 antibody, in the above example are 60% and 97.4%, respectively, using a biotinylated anti-human CD3 antibody and EasySep™ Human Biotin Positive Selection Kit.

STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485. PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED.

Copyright © 2017 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, EasySep, RoboSep, and SepMate are trademarks of STEMCELL Technologies Canada Inc. Lymphoprep is a trademark of AXIS-SHIELD. All other trademarks are the property of their respective holders. Users of this kit should ensure that they are entitled to use the antibody of interest. STEMCELL Technologies Inc. is not responsible for patent infringements or violations that may occur when using this product. 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.