EasySep™ Biotin Positive Selection Kit II

Positive Selection

Catalog #17683

For processing 1 x 10^9 cells

STEMCELL™ TECHNOLOGIES
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 #DX22366 | Version 1_0_0

Description

Isolate highly purified cells labeled with biotinylated antibodies from any single-cell suspension 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. 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™ Dextran RapidSpheres™ 50100	50100	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.
RoboSep™ Vial For Primary Conjugated Antibody	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

Prepare a single-cell suspension.

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 Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Biotin Positive Selection 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.**	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. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Add species-specific FcR blocker (not provided) to sample.**	0.5 - 3 µg/mL of sample	0.5 - 3 µg/mL of sample
3	**Add biotinylated antibody to sample.†**	0.3 - 3 µg/mL of sample	0.3 - 3 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 in step 1.	Top up with 10-fold excess recommended medium and centrifuge. Carefully aspirate and discard supernatant. Resuspend in the same volume as in 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	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
6	**Add RapidSpheres™ 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	• 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 **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.
§ Magnetic particles may be titrated to optimize performance; a range of 25 - 75 µL/mL is recommended.
‡ Purity may be improved by decreasing magnetic particle incubation time to 5 minutes.
* 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.

Table 2. EasySep™ Biotin Positive Selection Kit II Protocol

		EASYSEP™ MAGNETS	
		EasyEights™ (Catalog #18103)	
STEP	INSTRUCTIONS	5 mL tube	14 mL tube
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 0.1 - 2.5 mL	1 x 10^8 cells/mL 0.25 - 8 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 species-specific FcR blocker (not provided) to sample.	0.5 - 3 µg/mL of sample	0.5 - 3 µg/mL of sample
3	Add biotinylated antibody to sample.†	0.3 - 3 µg/mL of sample	0.3 - 3 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 in step 1.	Top up with 10-fold excess recommended medium and centrifuge. Carefully aspirate and discard supernatant. Resuspend in the same volume as in 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	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add RapidSpheres™ to sample.	75 µL/mL of sample§	75 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	• 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 10 minutes	RT for 10 minutes
8	Carefully pipette*** (do not pour) off the supernatant. Remove the tube, containing the isolated cells, from the magnet.	Discard supernatant	Discard supernatant
9	Repeat steps as indicated.	Steps 7 and 8, two more times (total of 3 x 10-minute separations)	Steps 7 and 8, two more times (total of 3 x 10-minute separations)
Continue on the next page.		Continue on the next page.	Continue on the next page.

STEP	INSTRUCTIONS (CONTINUED)	EASYSEP™ MAGNETS EasyEights™ (Catalog #18103) 5 mL tube	EASYSEP™ MAGNETS EasyEights™ (Catalog #18103) 14 mL tube
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 10-minute separations)	Repeat steps 7 and 8, up to three more times (total of 4 - 6 x 10-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.
§ Magnetic particles may be titrated to optimize performance; a range of 50 - 100 µL/mL is recommended.
‡ Purity may be improved by decreasing magnetic particle incubation time to 5 minutes.
*** Collect the entire supernatant, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube use a 10 mL serological pipette [Catalog #38004]).

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ Biotin Positive Selection Kit II 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 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Add species-specific FcR blocker (not provided) to sample and mix.**	0.5 - 3 µg/mL of sample
3	**Select protocol.**	Any Species Biotin Positive Selection 17683
4	**Transfer biotinylated antibody to the RoboSep™ Vial provided.**	Use of this vial is required for RoboSep™ to run properly
5	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
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

FcR BLOCKING ANTIBODY (NOT PROVIDED)

The FcR blocking antibody is used to prevent non-specific selection of monocytes and macrophages. A species-specific FcR blocking antibody may be required to achieve desired purities.

OPTIMIZING PURITY

Purity can be increased, for some cell types, by decreasing the amount of 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 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 makers.
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).