EasySep™ Mouse Biotin Positive Selection Kit

Positive Selection

Catalog #18556

For processing 1 x 10^9 cells

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Description

Isolate highly purified cells labeled with biotinylated antibodies from mouse splenocytes, bone marrow, 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. 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.
EasySep™ Mouse FcR Blocker	18720	1 x 0.1 mL	Store at 2 - 8°C. Do not freeze.	Stable for 1 year from date of receipt.	A combination of monoclonal antibodies in PBS, 0.1% BSA, and 0.1% sodium azide.
RoboSep™ Vial	18550	1 vial	Not applicable	Not applicable	Not applicable

BSA - bovine serum albumin; PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Please refer to the Safety Data Sheet (SDS) for hazard information.

Sample Preparation

SPLEEN

Disrupt spleen in PBS or Hanks' Balanced Salt Solution containing 2% fetal bovine serum (FBS). Remove clumps and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge at 300 x g for 10 minutes and resuspend at 1 x 10^8 nucleated cells/mL in recommended medium.

Ammonium chloride treatment is not recommended when preparing the cells for separation.

BONE MARROW

Flush bone marrow cells from femur and tibia into recommended medium using a syringe equipped with a 23 gauge needle. Disperse aggregates by gently passing the cell suspension through the syringe several times. Alternatively, crush bones using a mortar and pestle. Remove remaining aggregates and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge at 300 x g for 10 minutes and resuspend cells at 1 x 10^8 cells/mL in 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 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™ Mouse 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. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Add FcR blocker to sample and mix.**	10 µL/mL of sample	10 µL/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 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	• 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.

STEP	INSTRUCTIONS (CONTINUED)	EASYSEP™ MAGNETS  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.
* 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™ Mouse 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 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Add FcR blocker to sample and mix.**	10 µL/mL of sample
3	**Select protocol.**	Mouse Biotin Positive Selection 18556-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

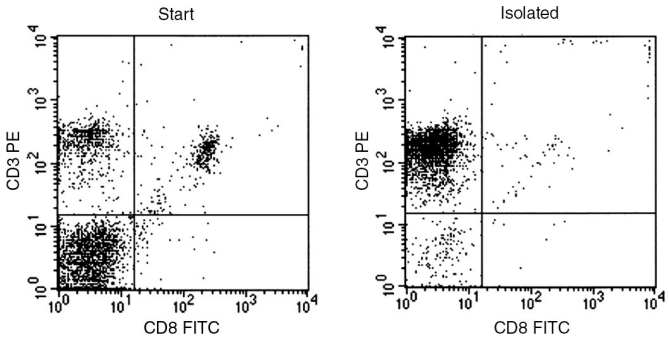
Recovery of positively selected biotin-labeled cells is dependent on the quality of the biotinylated antibody used. Antibodies that have expired or that have been stored improperly may show lower affinity for the surface marker on the target cell, resulting in lower recovery.

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 mouse splenocytes, the purities of the start and final isolated fractions (CD3+CD8-) in the above example are 22.8% and 93.9%, respectively, using a biotinylated anti-mouse CD4 antibody and EasySep™ Mouse Biotin Positive Selection Kit.