EasySep™ Mouse PE Positive Selection Kit II

Catalog #17666 For processing 1 x 10^9 cells
Catalog #17696 For processing 5 x 10^9 cells

Positive Selection

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Description

Isolate highly purified cells labeled with PE (phycoerythrin)-conjugated 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 wth PE-conjugated 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™ PE Selection Cocktail	18151	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™ 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.
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

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.

Sample Preparation

SPLEEN

Disrupt spleen in PBS or Hanks' Balanced Salt Solution containing 2% fetal bovine serum (FBS). Remove aggregates 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 nucleated cells/mL in recommended medium.

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

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™ Mouse PE 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 FcR blocker to sample and mix.	10 µL/mL of sample	10 µL/mL of sample
3	Add PE-conjugated 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 at 300 x g for 10 minutes at RT with low brake. Carefully aspirate and discard supernatant. Resuspend in the same volume as in step 1. NOTE: If needed, the wash step can be performed in a larger tube. Once resuspended, return sample to required tube as in step 1.	Top up with 10-fold excess recommended medium and centrifuge at 300 x g for 10 minutes at RT with low brake. Carefully aspirate and discard supernatant. Resuspend in the same volume as in step 1. NOTE: If needed, the wash step can be performed in a larger tube. Once resuspended, return sample to required tube as in step 1.
4	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	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 the next page.		Continue on the next page.	Continue on the next page.

STEP	INSTRUCTIONS (CONTINUED)	EASYSEP™ MAGNETS  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 PE-conjugated 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™ Mouse PE Positive Selection Kit II Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)	
		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 FcR blocker to sample and mix.	10 µL/mL of sample	10 µL/mL of sample
3	Add PE-conjugated 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 at 300 x g for 10 minutes at RT with low brake. Carefully aspirate and discard supernatant. Resuspend in the same volume as in step 1. NOTE: If needed, the wash step can be performed in a larger tube. Once resuspended, return sample to required tube as in step 1.	Top up with 10-fold excess recommended medium and centrifuge at 300 x g for 10 minutes at RT with low brake. Carefully aspirate and discard supernatant. Resuspend in the same volume as in step 1. NOTE: If needed, the wash step can be performed in a larger tube. Once resuspended, return sample to required tube as in step 1.
4	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	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 PE-conjugated 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™ Mouse PE Positive Selection Kit II Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #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 PE Positive Selection 17666
4	Transfer PE-conjugated 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

OPTIMIZING PURITY

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

OPTIMIZING RECOVERY

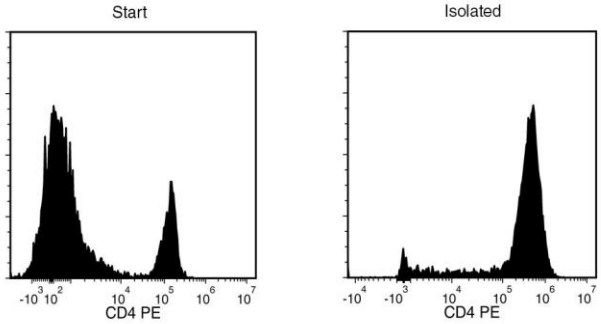
Recovery of positively selected PE-labeled cells is dependent on the quality of the PE-conjugated antibody used. Antibodies that have expired or that have been stored improperly may show lower affinity for the surface marker on the target cell, resulting in lower recovery.

It is important to add enough PE-conjugated antibody to ensure a significant fluorescence intensity of the target cells, as there is a strong correlation between fluorescence intensity and cell recovery. We recommend that the fluorescence intensity of the positively selected cells be at least 100-fold (2 logarithms) greater than that of the negative control for adequate recovery.

ASSESSING PURITY

The positively selected cells have already been PE-labeled, so the purity can be assessed directly by flow cytometry.

Data



Starting with mouse splenocytes, the purities of the start and final isolated fractions in the above example are 20.5% and 91.6%, respectively, using a PE-conjugated anti-mouse CD4 antibody and EasySep™ Mouse PE Positive Selection Kit II.