EasySep™ Mouse CD11b Positive Selection Kit

Positive Selection

Catalog #18770

For processing 2 x 10^9 cells

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Description

Isolate highly purified CD11b+ cells from mouse splenocytes by immunomagnetic positive selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Fast and easy-to-use
- Up to 96% purity
- No columns required

This kit targets CD11b+ cells for positive selection with an antibody recognizing the CD11b surface marker. 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, culture, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse CD11b PE Labelling Reagent	18770C	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, 0.1% BSA and < 0.1% sodium azide. Includes and Fc receptor blocking antibody.
EasySep™ PE Selection Cocktail	18151	2 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.

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 recommended medium. Remove aggegates 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.

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™ Mouse CD11b 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	1 x 10^8 cells/mL 0.25 - 6 mL NOTE: If starting with fewer than 2.5 x 10^7 cells, resuspend cells in 0.25 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 Labeling Reagent to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 15 minutes, protect from light	RT for 15 minutes, protect from light
3	Add Selection Cocktail to sample.	70 µL/mL of sample	70 µL/mL of sample
	Mix and incubate.	RT for 15 minutes, protect from light	RT for 15 minutes, protect from light
4	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
5	Add Magnetic Particles to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes, protect from light	RT for 10 minutes, protect from light
6	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 < 1.5 mL • Top up to 10 mL for samples ≥ 1.5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
7	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
8	Repeat steps as indicated.	Steps 6 and 7, two more times (total of 3 x 5-minute separations)	Steps 6 and 7, two more times (total of 3 x 5-minute separations)
OPTIONAL ADDITIONAL SEPARATION For samples ≥ 1 mL an additional separation is recommended.		Steps 6 and 7 (total of 4 x 5-minute separations)	Steps 6 and 7 (total of 4 x 5-minute separations)
9	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)

* 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 CD11b Positive Selection Kit Protocol

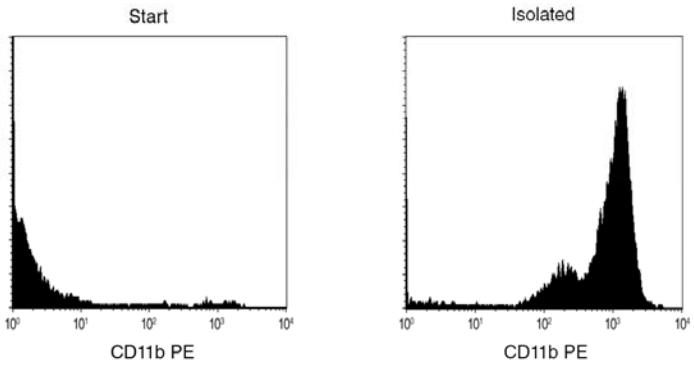
STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	**Prepare sample at the indicated cell concentration within the volume range.**	1 x 10^8 cells/mL 0.25 - 6 mL NOTE: If starting with fewer than 2.5 x 10^7 cells, resuspend cells in 0.25 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	**Select protocol.**	• For sample volumes between 0.25 - ≤ 5 mL: Mouse CD11b Positive Selection 18770 - small volume • For sample volumes between > 5 - 6 mL: Mouse CD11b Positive Selection 18770 - large volume
3	**Mix Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	Pipette up and down more than 5 times
4	**Load the carousel.**	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
5	**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

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 CD11b+ cell content of the isolated fraction typically ranges from 92 - 96%. In the above example, the purities of the start and final isolated fractions are 7.15% and 94.06%, respectively.