



EasySep™ Mouse CD11c Positive Selection Kit

Positive Selection

Catalog #18758

#18768

For processing 2×10^9 cells

For processing 2×10^9 cells, includes
10 x 4 mL Spleen Dissociation Medium



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Description

Isolate highly purified CD11c+ cells from mouse splenocytes or other single-cell suspensions 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 98% purity
- No columns required

This kit targets CD11c+ cells for positive selection with an antibody recognizing the CD11c 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

Spleen Dissociation Medium is sold as part of Catalog #18768 or is available for individual sale.

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse CD11c PE Labeling Reagent	18758C.2	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 with 0.1% BSA and < 0.1% sodium azide.
EasySep™ PE Selection Cocktail	18154	3 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 with 0.1% BSA.
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	18730	1 x 0.2 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS with 0.1% BSA and < 0.1% sodium azide.
Spleen Dissociation Medium*	07915	10 x 4 mL	Store at -20°C.	Stable until expiry date (EXP) on label.	Contains collagenase IV, DNase, FBS, and RPMI medium.

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

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

Sample Preparation

SPLEEN

Use Spleen Dissociation Medium (Catalog #07915). For the recommended protocol, refer to the Dissociation at Room Temperature (15 - 25°C) in the Product Information Sheet for Spleen Dissociation Medium (Document #29636).

After processing, resuspend at 1×10^8 cells/mL (EasySep™) or 1.5×10^8 cells/mL (RoboSep™) in recommended medium.

BONE MARROW

Flush bone marrow cells from femur and tibia into recommended medium using a syringe equipped with a 23 gauge needle. Disperse clumps by gently passing the cell suspension through the syringe several times. Alternatively, crush bones using a mortar and pestle. Remove remaining clumps and debris by passing cell suspension through a 70 µm mesh nylon strainer.

Centrifuge at $300 \times g$ for 10 minutes and resuspend cells at $0.5 - 2 \times 10^6$ cells/mL in RPMI 1640 Medium (Catalog #36750) containing 10% FBS, 2 mM L-Glutamine (Catalog #07100), 5 µM 2-mercaptoethanol, 10 ng/mL Mouse Recombinant GM-CSF (Catalog #78017), and either 10 ng/mL Mouse Recombinant IL-4 (Catalog #78047) or 200 ng/mL Mouse Recombinant Flt3/Flk-2 Ligand (Catalog #78011).

Plate 20 mL per 150 mm Petri dish and incubate at 37°C for 5 days. Collect non-adherent cells, wash once, and resuspend at 1×10^8 cells/mL (EasySep™) or 1.5×10^8 cells/mL (RoboSep™) 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 CD11c 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 mL NOTE: If starting with fewer than 1 x 10 ⁷ cells, resuspend cells in 0.1 mL	1 x 10 ⁸ cells/mL 0.5 - 6 mL NOTE: If starting with fewer than 5 x 10 ⁷ cells, resuspend cells in 0.5 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.†	10 µL/mL of sample	10 µL/mL of sample
3	Add Labeling Reagent to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 15 minutes	RT for 15 minutes
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 < 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the tube (without lid) into the magnet and incubate.	<ul style="list-style-type: none"> • For spleen cells: RT for 5 minutes • For cultured cells: RT for 10 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.	<ul style="list-style-type: none"> • For spleen cells: Steps 7 and 8, three more times (total of 4 x 5-minute separations) • For cultured cells: Steps 7 and 8, one more time (total of 2 x 10-minute separations) 	<ul style="list-style-type: none"> • For spleen cells: Steps 7 and 8, three more times (total of 4 x 5-minute separations) • For cultured cells: Steps 7 and 8, one more time (total of 2 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)


† Using an FcR blocker may prevent downstream attempts at cross-linking FcγRIII/II (CD16/CD32) molecules and triggering signaling through these receptors (Siragam et al.). The EasySep™ Mouse FcR Blocker may be omitted if necessary.

* 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 CD11c Positive Selection Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000)	
1	Prepare sample at the indicated cell concentration within the volume range.	1.5 x 10 ⁸ cells/mL 0.3 - 4.5 mL NOTE: If starting with fewer than 5 x 10 ⁷ cells, resuspend cells in 0.3 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.†	10 µL/mL of sample	
3	Select protocol.	<u>Spleen</u> <ul style="list-style-type: none"> For samples ≤ 3 mL use: Mouse CD11c Positive Selection (spleen DC) 18758 - small volume For samples ≥ 3 mL use: Mouse CD11c Positive Selection (spleen DC) 18758 - large volume <u>Cultured Bone Marrow</u> <ul style="list-style-type: none"> For samples ≤ 3 mL use: Mouse CD11c Positive Selection (cultured DC) 18758 - small volume For samples ≥ 3 mL use: Mouse CD11c Positive Selection (cultured DC) 18758 - large volume 	
4	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times	
5	Load the carousel.	Follow on-screen prompts	
	Start the protocol.	Press the green "Run" button	
6	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	

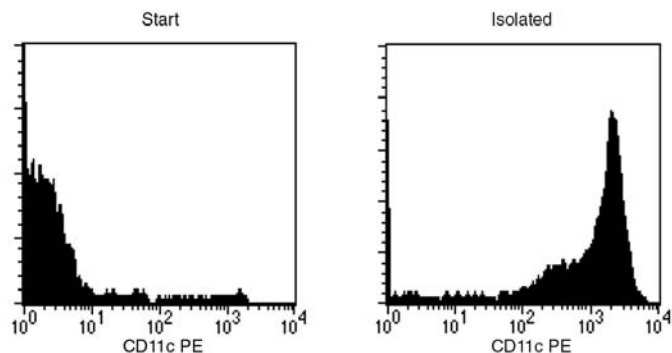
† Using an FcR blocker may prevent downstream attempts at cross-linking FcγRIII/II (CD16/CD32) molecules and triggering signaling through these receptors (Siragam et al.). The EasySep™ Mouse FcR Blocker may be omitted if necessary.

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 CD11c+ cell content of the isolated fraction typically ranges from 87 - 98%. In the above example, the purity of the start and final isolated fractions are 7.2% and 93.1%, respectively.

References

Siragam V et al. (2006) Intravenous immunoglobulin ameliorates ITP via activating Fc gamma receptors on dendritic cells. *Nat Med* 12(6): 688–92.

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