EasySep™ Positive Selection

EasySep™ Mouse CD11b Positive Selection Kit II

Catalog #18970

For processing 2 x 10^9 cells from lung tissue

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

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Description

Isolate highly purified CD11b+ cells from mouse lung tissues by positive selection.

- Fast and easy-to-use
- Up to 95% purity
- No columns required
- Isolated cells are not fluorochrome-labeled

This kit targets CD11b+ cells for positive selection with antibodies 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, cell culture, and cell-based experiments.

- This is the Product Information Sheet (PIS) for isolating CD11b+ cells from mouse lungs. If isolating CD11b+ cells from mouse splenocytes or bone marrow samples, refer to the applicable PIS (Document #DX20430).
- If isolating CD11b+ cells from mouse brain tissues, refer to the applicable PIS (Document #DX21971).

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse CD11b Positive Selection II Component A	18970CA	1 x 0.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS and 0.1% BSA.
EasySep™ Mouse CD11b Positive Selection II Component B	18970CB	1 x 0.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS and 0.1% BSA.
EasySep™ Dextran RapidSpheres™ 50100	50100	2 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™ Empty Vial	27401	1	Not applicable	Not applicable	Not applicable
Normal Rat Serum	13551	1 x 2 mL	Store at -20°C.	Stable until expiry date (EXP) on label.	Mycoplasma-free normal rat serum.

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.

Additional Reagent Stability Information

REAGENT NAME	STORAGE	SHELF LIFE
Selection Cocktail (combined Component A + Component B)	Store at 2 - 8°C. Do not freeze.	Stable for up to 4 weeks. Do not exceed the expiry date (EXP) of individual components.
Normal Rat Serum (in-use)	Store at 2 - 8°C.	Stable for at least 2 months. Do not exceed the expiry date (EXP) on label.

Sample Preparation

LUNG TISSUE

1. Prepare lung digestion medium and warm to room temperature (15 - 25°C). To prepare lung digestion medium, combine the following:
   - Liberase™ TM Research Grade (Sigma-Aldrich, Catalog #5401119001) to a final concentration of 0.25 mg/mL
   - DNase I Solution (1 mg/mL; Catalog #07900) to a final concentration of 250 µL/mL
   - RPMI 1640 Medium (Catalog #36750) to make up the remaining volume
2. Harvest lung tissue into a 50 mL conical tube rinse with PBS or PBS containing 2% fetal bovine serum (FBS).
3. Transfer lung tissue into a dish without medium. Mince into a homogenous paste (< 1 mm in size) using a razor blade or scalpel.
4. Transfer minced lung tissue into a tube containing lung digestion medium and incubate at 37°C for 30 minutes on a shaking platform.
   NOTE: Use 2 mL of lung digestion medium for up to 4 lungs. For more than 4 lungs, use 0.5 mL of lung digestion medium per lung.
5. Using a syringe equipped with a 20 gauge needle, disperse aggregates by gently passing the digested lung tissue through the syringe several times.
6. Place a 70 µm nylon mesh strainer over a 50 mL conical tube and rinse with recommended medium. Transfer the digested lung tissue into the strainer and push the tissue through strainer with the rubber end of a syringe plunger to obtain a cell suspension. Rinse the strainer with recommended medium. Use new strainers as necessary.
7. Centrifuge at 300 x g for 10 minutes at room temperature with the brake on low. Carefully remove and discard the supernatant.
8. Add 20 mL of Ammonium Chloride Solution (Catalog #07800) to the cell pellet. Incubate for 10 - 15 minutes on ice.
9. Top up to 50 mL with recommended medium. Centrifuge at 300 x g for 10 minutes at room temperature with the brake on low. Carefully remove and discard the supernatant.
10. Resuspend cells at 5 x 10^7 cells/mL in recommended medium.

SPLEEN OR BONE MARROW

If processing spleen or bone marrow samples, refer to the applicable PIS (Document #DX20430).

BRAIN TISSUE

If processing brain tissue, refer to the applicable PIS (Document #DX21971).

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

Table 1. EasySep™ Mouse CD11b Positive Selection Kit II Protocol for LUNG TISSUE

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 0.2 - 1.5 mL	5 x 10^7 cells/mL 0.2 - 3 mL
2	Add Rat Serum to sample.	25 µL/mL of sample	25 µL/mL of sample
3	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)
4	Prepare Selection Cocktail in a tube. For each 1 mL of sample make 25 µL of cocktail (12.5 µL of Component A + 12.5 µL of Component B).	Mix equal volumes of Component A and Component B. Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.	Mix equal volumes of Component A and Component B. Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.
	Incubate.	RT for 5 minutes	RT for 5 minutes
5	Add Selection Cocktail to sample.	25 µL/mL of sample	25 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
6	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
7	Add RapidSpheres™ to sample.	40 µL/mL of sample	40 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
8	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 3 minutes	RT for 5 minutes
9	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
10	Repeat steps as indicated.	Steps 8 and 9, four more times (total of 5 x 3-minute separations)	Steps 8 and 9, three more times (total of 4 x 5-minute separations)
11	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.

Table 2. EasySep™ Mouse CD11b Positive Selection Kit II Protocol for LUNG TISSUE

		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.	5 x 10^7 cells/mL 0.2 - 1 mL	5 x 10^7 cells/mL 0.5 - 3 mL
2	Add Rat Serum to sample.	25 µL/mL of sample	25 µL/mL of sample
3	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)
4	Prepare Selection Cocktail in a tube. For each 1 mL of sample make 25 µL of cocktail (12.5 µL of Component A + 12.5 µL of Component B).	Mix equal volumes of Component A and Component B. Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.	Mix equal volumes of Component A and Component B. Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.
	Incubate.	RT for 5 minutes	RT for 5 minutes
5	Add Selection Cocktail to sample.	35 µL/mL of sample	35 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
6	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
7	Add RapidSpheres™ to sample.	60 µL/mL of sample	60 µL/mL of sample
	Mix and incubate.	RT for 3 minutes	RT for 3 minutes
8	Add recommended medium to top up 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 < 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes
9	Carefully pipette** (do not pour) off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
10	Add recommended medium to top up 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 < 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
11	Repeat steps as indicated.	Steps 9 and 10, two more times (total of 1 x 10-minute and 3 x 5-minute separations)	Steps 9 and 10 (total of 1 x 10-minute and 2 x 5-minute separations)
12	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)

** 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 CD11b Positive Selection Kit II Protocol for LUNG TISSUE

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 0.25 - 3 mL
	Add Rat Serum to sample.	25 µL/mL of sample
3	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38007)
4	Prepare Selection Cocktail in the RoboSep™ Empty Vial provided. See Table 4 for required volumes.	Mix equal volumes of Component A and Component B (see Table 4). Prepared cocktail is stable at 2 - 8°C for up to 4 weeks.
	Incubate.	RT for 5 minutes
5	Select protocol.	Mouse CD11b Positive Selection II 18970 Lung
6	Vortex RapidSpheres™.	30 seconds
7	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
8	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

Table 4. RoboSep™ Selection Cocktail Preparation

START SAMPLE	COMPONENT A	COMPONENT B	SELECTION COCKTAIL TOTAL VOLUME
1 mL	75 µL	75 µL	150 µL
1.5 mL	87.5 µL	87.5 µL	175 µL
2 mL	100 µL	100 µL	200 µL
3 mL	125 µL	125 µL	250 µL
4 mL	150 µL	150 µL	300 µL

Note: RoboSep™ requires an excess of the Selection Cocktail to run properly (as indicated above).

Notes and Tips

ASSESSING PURITY

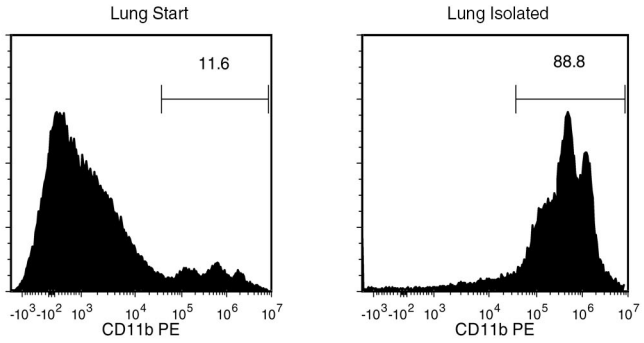
For purity assessment by flow cytometry use the following fluorochrome-conjugated antibody clone:

- Anti-Mouse CD11b Antibody, Clone M1/70 (Catalog #60001) at a concentration of 5 µg/mL

The following methods can also be used:

- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).
- Add fluorochrome-conjugated Anti-Mouse CD11b Antibody, Clone M1/70 at a concentration of 0.5 µg/mL immediately after adding the cocktail. This method labels the positive cells in the entire sample.

Data



Starting with mouse lung single-cell suspension, the CD11b+ cell content of the isolated fraction is typically 86.9 ± 7.6% (mean ± SD using the purple EasySep™ Magnet).