EasySep™ Mouse F4/80 Positive Selection Kit

For processing 7.5 x 10^8 cells from lung tissue

Catalog #100-0659

Positive Selection

Document #10000010446 | Version 00

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Description

Isolate highly purified F4/80+ 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 F4/80+ cells for positive selection with antibodies recognizing the F4/80 cell 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.

NOTE: This is the Product Information Sheet (PIS) for isolating F4/80+ cells from mouse lung tissue. If isolating F4/80+ cells from mouse splenocytes or peritoneal lavage, refer to the applicable PIS, available at www.stemcell.com or contact us to request a copy.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse F4/80 Positive Selection Component A	300-0251	1 x 0.3 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 F4/80 Positive Selection Component B	300-0253	1 x 0.6 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS with 5% HPCD.
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.
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; HPCD - 2-hydroxypropyl-β-cyclodextrin; 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
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

The following instructions are for processing 5 - 10 mouse lungs. If starting with more than 10 lungs, adjust volumes accordingly.

1. Prepare 10 mL of lung digestion medium by adding 1 mL of Collagenase/Hyaluronidase (Catalog #07912) and 1.5 mL of DNase I Solution (1 mg/mL; Catalog #07900) to 7.5 mL of RPMI 1640 Medium (Catalog #36750). Warm to room temperature (15 - 25°C).
2. Harvest lung tissue into a 50 mL conical tube with PBS containing 2% fetal bovine serum (FBS).
3. Transfer lung tissue to a new 50 mL conical tube containing 10 mL of digestion medium. Using scissors, mince the tissue into small pieces. Incubate at 37°C for 20 minutes on a shaking platform.
4. Place a 70 µm nylon mesh strainer (e.g. Catalog #27260) in a 100 mm dish (e.g. Catalog #38045) and push the digested lung tissue through the strainer with the rubber end of a syringe plunger to obtain a cell suspension.
5. Place a new 70 µm nylon mesh strainer over a 50 mL conical tube and filter the cell suspension through the strainer. Rinse the strainer with recommended medium and collect in the same tube.
6. Centrifuge at 300 x g for 10 minutes at room temperature with the brake on low. Carefully remove and discard the supernatant.
7. Add 20 mL of Ammonium Chloride Solution (Catalog #07800) to the cell pellet. Incubate at room temperature for 5 minutes.
8. 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.
9. Resuspend cells at 5 x 10^7 nucleated cells/mL in recommended medium.

SPLEEN OR PERITONEAL LAVAGE

If processing spleen or peritoneal lavage, refer to the applicable PIS, available at www.stemcell.com or contact us to request a copy.

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 2 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 F4/80 Positive Selection Kit 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 0.1 - 2 mL	5 x 10^7 cells/mL 0.5 - 8 mL
2	**Add Rat Serum to sample.**	50 µL/mL of sample	50 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 60 µL of cocktail (20 µL of Component A + 40 µL of Component B).**	Mix Component A and Component B at a 1:2 ratio. NOTE: Selection Cocktail must be prepared fresh before each use.	Mix Component A and Component B at a 1:2 ratio. NOTE: Selection Cocktail must be prepared fresh before each use.
	Incubate.	RT for 5 minutes	RT for 5 minutes
5	**Add Selection Cocktail to sample.** **NOTE: Do not vortex cocktail.**	60 µL/mL of sample	60 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 5 minutes	RT for 5 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 ≤ 3 mL • Top up to 10 mL for samples > 3 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 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, two more times (total of 3 x 5-minute separations)	Steps 8 and 9, two more times (total of 3 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 F4/80 Positive Selection Kit Protocol for LUNG TISSUE

		EASYSEP™ MAGNETS		
STEP	INSTRUCTIONS	 EasyPlate™ (Catalog #18102)	 EasyEights™ (Catalog #18103) 5 mL tube	EasyEights™ (Catalog #18103) 14 mL tube 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 0.1 - 0.2 mL	5 x 10^7 cells/mL 0.25 - 2 mL	5 x 10^7 cells/mL 0.5 - 8 mL
2	Add Rat Serum to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
3	Add sample to required tube or plate.	Round-bottom, non-tissue culture-treated 96-well plate (e.g. Catalog #38018)	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 60 µL of cocktail (20 µL of Component A + 40 µL of Component B).	Mix Component A and Component B at a 1:2 ratio. NOTE: Selection Cocktail must be prepared fresh before each use.	Mix Component A and Component B at a 1:2 ratio. NOTE: Selection Cocktail must be prepared fresh before each use.	Mix Component A and Component B at a 1:2 ratio. NOTE: Selection Cocktail must be prepared fresh before each use.
	Incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
5	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	60 µL/mL of sample	60 µL/mL of sample	60 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
6	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
7	Add RapidSpheres™ to sample.	60 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 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 0.25 mL	Top up to 2.5 mL	• Top up to 5 mL for samples ≤ 3 mL • Top up to 10 mL for samples > 3 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 10 minutes	RT for 10 minutes
9	Carefully pipette** (do not pour) off the supernatant. Remove the tube or plate from the magnet; this tube or plate contains the isolated cells.	Discard supernatant	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 0.25 mL	Top up to 2.5 mL	• Top up to 5 mL for samples ≤ 3 mL • Top up to 10 mL for samples > 3 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
11	Carefully pipette** (do not pour) off the supernatant. Remove the tube or plate from the magnet; this tube or plate contains the isolated cells.	Discard supernatant	Discard supernatant	Discard supernatant
12	Repeat steps as indicated.	Steps 10 and 11 (total of 3 x 5-minute separations)	Steps 10 and 11 (total of 1 x 10-minute and 2 x 5-minute separations)	Steps 10 and 11 (total of 1 x 10-minute and 2 x 5-minute separations)
13	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube or plate.	Isolated cells are ready for use	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]).

Notes and Tips

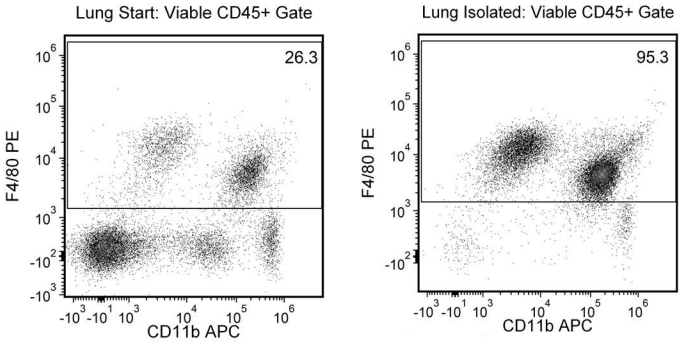
ASSESSING PURITY

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

- Anti-Mouse F4/80 Antibody, Clone BM8 (Catalog #60027), and
- Anti-Mouse CD11b Antibody, Clone M1/70 (Catalog #100-0433), and
- Anti-Mouse CD45 Antibody, Clone 30-F11 (Catalog #60030)

NOTE: To exclude dead cells for purity assessment, use of the nuclear dye DRAQ7™ is recommended.

Data



Starting with a naïve mouse lung single-cell suspension, the F4/80+ cell content of the isolated fraction is typically 94.3 ± 2.8% (mean ± SD using the purple EasySep™ magnet). In the above example, the purities of the start and final isolated fractions are 26.3% and 95.3%, respectively.