EasySep™ Mouse CD11b Positive Selection Kit II

For processing 7 x 10^8 cells from brain tissue

Catalog #18970

Positive Selection

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Description

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

- Fast, easy-to-use, and column-free
- Up to 98% purity
- 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.

NOTE: This is the Product Information Sheet (PIS) for isolating CD11b+ cells from mouse brain tissues. If isolating CD11b+ cells from mouse spleen, bone marrow, or lung tissue, 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 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
Mouse FcR PolyBlock*	300-0902	1 x 1.2 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of polyclonal antibodies and maltose in water with 5 µg/mL Triton X-100.

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

* Not required to isolate CD11b+ cells from brain tissue

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.

Sample Preparation

BRAIN TISSUE

1. Prepare sample preparation medium as follows:
   - DMEM/F-12 with 15 mM HEPES (Catalog #36254) containing 2% fetal bovine serum (FBS), OR
   - HBSS, Modified (Without Ca++ and Mg++; Catalog #37250) containing 2% FBS and 1 mM EDTA
2. Prepare brain digestion medium by combining the following:
   - Papain (Catalog #07465) to a final concentration of 20 units/mL
   - DNase I Solution (1 mg/mL; Catalog #07900) to a final concentration of 100 µg/mL
   - HBSS, Modified (Without Ca++ and Mg++) or DMEM/F-12 with 15 mM HEPES to make up the remaining volume

   Warm medium to room temperature (15 - 25°C) before use.

   NOTE: For up to three brains, prepare 3 mL of brain digestion medium. For four or more brains, prepare 1 mL of brain digestion medium per brain.
3. Add at least 1 mL of brain digestion medium to a Petri dish (e.g. Catalog #27110).
4. Harvest brains and place in the Petri dish prepared in step 3. Use scissors, scalpel, or razor blade to mince brains into small pieces (< 1 mm).
5. Transfer the minced brain tissue to a 50 mL conical tube (e.g. Catalog #38010). Rinse the dish with the remaining brain digestion medium and add to the 50 mL conical tube. Incubate at 37°C for 30 minutes on a shaking platform.
6. Place a 70 µm nylon mesh strainer (e.g. Catalog #27260) over a new 50 mL conical tube and rinse with sample preparation medium (prepared in step 1). Transfer the digested brain 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 sample preparation medium. Use new strainers as necessary.
7. Centrifuge at 300 x g for 10 minutes at room temperature or at 2 - 8°C, with the brake on low. Using a serological pipette, carefully remove and discard the supernatant.
8. Prepare 6 mL of 30% Percoll® solution per brain by combining 3 parts 100% isotonic Percoll® with 7 parts sample preparation medium.

   NOTE: To make 100% isotonic Percoll® solution, combine 9 parts Percoll® (GE Healthcare Catalog #17-0891-01) with 1 part D-PBS, 10X Concentrate (Without Ca++ and Mg++; Catalog #37354).
9. Add 30% Percoll® solution (prepared in step 8) to the pellet.

   NOTE: For volumes > 30 mL, use 50 mL tubes; for volumes < 30 mL, use one or more 14 mL tubes).
10. Centrifuge at 700 x g for 10 minutes at room temperature or at 2 - 8°C with the brake off.
11. Using a 1 mL wide-bore pipette tip or a 2 mL serological pipette (e.g. Catalog #38002), carefully remove and discard the upper myelin layer.
12. Using a serological pipette, remove and discard the remaining supernatant.
13. Transfer the cells to a new tube. Top up with sample preparation medium. Centrifuge at 300 x g for 10 minutes at room temperature or at 2 - 8°C with the brake on low. Carefully remove and discard the supernatant.
14. Count and resuspend cells at 2.5 x 10^7 cells/mL in recommended medium.

SPLEEN, BONE MARROW, OR LUNG TISSUE

If processing spleen, bone marrow, or lung tissue, refer to the applicable PIS, available at www.stemcell.com or contact us to request a copy.

Recommended Medium

EasySep™ Buffer (Catalog #20144) 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 CD11b Positive Selection Kit II Protocol for BRAIN 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.	2.5 x 10^7 cells/mL 0.1 - 1 mL	2.5 x 10^7 cells/mL 0.1 - 1 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	Prepare Selection Cocktail in a tube. For each 1 mL of sample make 35 µL of cocktail (17.5 µL of Component A + 17.5 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
3	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	35 µL/mL of sample	35 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
5	Add RapidSpheres™ to sample.	80 µL/mL of sample	80 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
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
	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)
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.

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

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasyPlate™ (Catalog #18102)**	**EasyEights™ (Catalog #18103) 5 mL tube** 
1	Prepare sample at the indicated cell concentration within the volume range.	2.5 x 10^7 cells/mL 0.05 - 0.2 mL	2.5 x 10^7 cells/mL 0.1 - 0.5 mL
	Add sample to required tube (or plate when using the EasyPlate™ EasySep™ Magnet).	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)
2	Prepare Selection Cocktail in a tube. For each 1 mL of sample make 35 µL of cocktail (17.5 µL of Component A + 17.5 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
3	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	35 µL/mL of sample	35 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
5	Add RapidSpheres™ to sample.	80 µL/mL of sample	80 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
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 0.25 mL	Top up to 2.5 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 10 minutes
7	Carefully pipette* (do not pour) off the supernatant. Remove the tube or plate, containing the isolated cells, from the magnet.	Discard supernatant	Discard supernatant
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 0.25 mL	Top up to 2.5 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
9	Carefully pipette* (do not pour) off the supernatant. Remove the tube or plate, containing the isolated cells, from the magnet.	Discard supernatant	Discard supernatant
10	Repeat steps as indicated.	Steps 8 and 9 (total of 3 x 5-minute separations)	Steps 8 and 9 (total of 1 x 10-minute and 2 x 5-minute separations)
11	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

RT - room temperature (15 - 25°C)

* Collect the entire supernatant, all at once, into a single pipette (e.g. for the EasyEights™ 5 mL tube, use a 2 mL serological pipette [Catalog #38002]).

Notes and Tips

ASSESSING PURITY

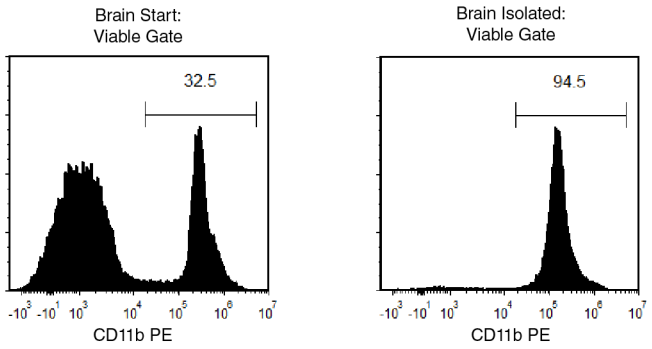
For purity assessment by flow cytometry use the following fluorochrome-conjugated antibody clone:

- 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 a single-cell suspension of mouse brain cells, the CD11b+ cell content of the isolated fraction is typically 94.2 ± 4.0% (mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 32.5% and 94.5%, respectively.