EasySep™ Mouse CD11c Positive Selection Kit

For processing 2 x 10^9 cells

Catalog #18780, #18780RF RoboSep™
#18781, #18781RF RoboSep™ Includes 10 x 4 mL Spleen Dissociation Medium

Negative Selection

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Description

Isolate highly purified CD11c+ cells from mouse splenocytes or cultured bone marrow cells by immunomagnetic positive selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Fast, easy-to-use, and column-free
- Up to 95% purity
- Isolated cells are not fluorochrome-labeled

This kit targets CD11c+ cells for positive selection with antibodies 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, and cell-based experiments.

Component Descriptions

Spleen Dissociation Medium is sold as part of Catalog #18781 and is also available for individual sale.

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse CD11c Positive Selection Kit II Component A	18780CA	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 CD11c Positive Selection Kit II Component B	18780CB	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.
EasySep™ Mouse FcR Blocker	18731	3 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, 0.1% BSA, and < 0.1% sodium azide.
RoboSep™ Empty Vial	27401	1	Not applicable	Not applicable	Not applicable
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.

Additional Reagent Stability Information

COMPONENT NAME	STORAGE	SHELF LIFE
Selection Cocktail (combined Component A + Component B)	Store at 2 - 8°C. Do not freeze.	Stable for up to 2 weeks. Do not exceed expiry date (EXP) on label of individual components.

Sample Preparation

SPLEEN

Use Spleen Dissociation Medium (Catalog #07915). For more information on the use of Spleen Dissociation Medium, refer to the applicable Product Information Sheet (Document #29636).

1. Incubate minced spleen in Spleen Dissociation Medium at room temperature (15 - 25°C) for 30 minutes.
2. Dissociate spleen fragments into a smooth suspension by gently passing several times through an 18 gauge needle attached to a 3 cc Syringe (Catalog #28230).
3. Pour the entire suspension through a pre-wetted 70 µm nylon mesh filter into a 50 mL conical screw-cap tube.
4. Rinse the empty Spleen Dissociation Medium tube and mesh filter with 10 mL of PBS containing 2% FBS without EDTA (e.g. Catalog #07905) and add to the 50 mL conical tube.
5. Centrifuge the 50 mL conical tube at 300 x g for 10 minutes and pour off the supernatant.
6. Resuspend the cell pellet in ~0.5 mL of PBS containing 2% FBS without EDTA per spleen.
7. Add DNase I Solution (Catalog #07900) to a final concentration of 100 µg/mL, and incubate at room temperature for 10 minutes.
8. Count cells and resuspend at 1 x 10^8 nucleated cells/mL in recommended medium (containing 1 mM EDTA). Ammonium chloride treatment is not recommended when preparing the cells for separation.

BONE MARROW

For protocols with culture-expanded bone marrow-derived dendritic cells, contact us at techsupport@stemcell.com.

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 CD11c Positive Selection Kit II 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 0.5 - 2 mL	1 x 10^8 cells/mL 1 - 4 mL
2	**Add FcR Blocker to sample.**	60 µL/mL of sample	60 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, prepare 50 µL of cocktail (25 µL of Component A + 25 µL of Component B).**	Mix equal volumes of Component A and Component B. Selection Cocktail is stable at 2 - 8°C for up to 2 weeks.	Mix equal volumes of Component A and Component B. Selection Cocktail is stable at 2 - 8°C for up to 2 weeks.
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	**Add Selection Cocktail to sample.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
6	**Vortex Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
7	**Add RapidSpheres™ to sample.**	40 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 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 ≤ 2 mL • Top up to 10 mL for samples ≥ 2 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 3 minutes	RT for 3 minutes
9	**Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.**	Discard supernatant	Discard supernatant
10	**Repeat steps as indicated.**	Steps 8 and 9, three more times (total of 4 x 3-minute separations)	Steps 8 and 9, three more times (total of 4 x 3-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 CD11 Positive Selection Kit II Protocol

		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.	1 x 10^8 cells/mL 0.25 - 1 mL	1 x 10^8 cells/mL 1 - 5 mL
2	Add FcR Blocker to sample.	60 µL/mL of sample	60 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, prepare 50 µL of cocktail (25 µL of Component A + 25 µL of Component B).	Mix equal volumes of Component A and Component B. Selection Cocktail is stable at 2 - 8°C for up to 4 weeks.	Mix equal volumes of Component A and Component B. Selection Cocktail is stable at 2 - 8°C for up to 4 weeks.
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	50 µL/mL of sample	50 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	Carefully pipette** (do not pour) off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	Discard supernatant
12	Repeat steps as indicated.	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	Carefully pipette** (do not pour) the enriched cell suspension into a new 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 2 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

Table 2. RoboSep™ Mouse Neutrophil Enrichment Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	**Prepare sample at the indicated cell concentration within the volume range.**	1 x 10^8 cells/mL 0.5 - 4 mL
2	**Add FcR Blocker to sample.**	60 µL/mL of sample
3	**Add sample to required tube.**	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
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). Selection Cocktail is stable at 2 - 8°C for up to 2 weeks.
	Incubate.	RT for 5 minutes
6	**Select protocol.**	Mouse CD11c Positive Selection II 18780v2
7	**Vortex Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
8	**Load the carousel.**	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
9	**Unload the carousel when the run is complete. Remove the tube containing the isolated cells.**	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

Table 4. RoboSep™ Selection Cocktail Preparation

START SAMPLE	COMPONENT A	COMPONENT B	SELECTION COCKTAIL TOTAL VOLUME
0.5 mL	62.5 µL	62.5 µL	125 µL
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 extra 100 µL of the Selection Cocktail to run properly (compared to manual protocols).

Notes and Tips

ASSESSING PURITY

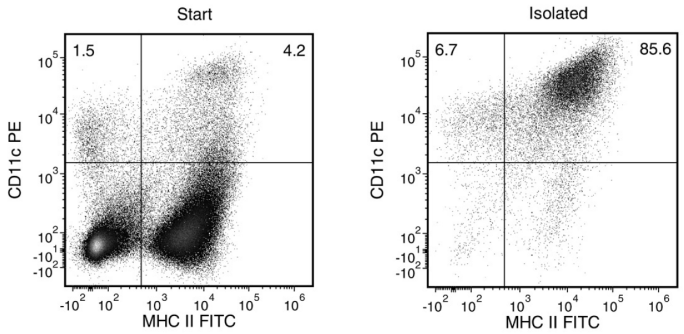
EasySep™ Mouse CD11c Positive Selection Cocktail uses an anti-CD11c antibody clone that to our knowledge partially blocks most anti-CD11c antibody clones used to assess purity by flow cytometry. For purity assessment of CD11c+ cells by flow cytometry, use the following method:

- Add fluorochrome-conjugated Anti-Mouse CD11c Antibody, Clone N418 (Catalog #60002) at a concentration of 0.4 µg/mL immediately after adding the cocktail. This method labels the positive cells in the entire sample.

One of the following methods can also be used:

- Use a fluorochrome-conjugated secondary antibody, such as goat anti-hamster IgG (H+L) antibody.
- Use Anti-Dextran Antibody, Clone DX1 (Catalog #60026), which recognizes the dextran on the EasySep™ Dextran RapidSpheres™.
- Use alternative markers for your cell type of interest, if applicable.

Data



Starting with mouse splenocytes, the CD11c+ cell content of the enriched fraction is typically 86.8 ± 9.7% (gated on viable singlet cells, mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 5.7% and 92.3%, respectively.