EasySep™ Mouse CD11c Positive Selection Kit II

Positive Selection

Catalog #18780 For processing 2 x 10^9 cells

#18781 For processing 2 x 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 cultured bone marrow cells 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 95% purity
- No columns required
- 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.
Normal Rat Serum	13551	1 x 2 mL	Store at -20°C.	Stable until expiry date (EXP) on label.	Mycoplasma-free normal rat serum.
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

REAGENT 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.
Normal Rat Serum (in-use)	Store at 2 - 8°C.	Stable for up to 2 months. Do not exceed expiry date (EXP) on label.

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 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 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.
	Incubate.	RT for 5 minutes	RT for 5 minutes
5	Add Selection Cocktail to sample.	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.	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 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, 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 CD11c Positive Selection Kit II Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	EasyEights™ (Catalog #18103)	
		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 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 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.
	Incubate.	RT for 5 minutes	RT for 5 minutes
5	Add Selection Cocktail to sample.	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	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 CD11c Positive Selection Kit II Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #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 Rat Serum to sample.**	50 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
5	**Select protocol.**	Mouse CD11c Positive Selection II 18780v2
6	**Vortex RapidSpheres™.** **NOTE: Particles should appear evenly dispersed.**	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

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

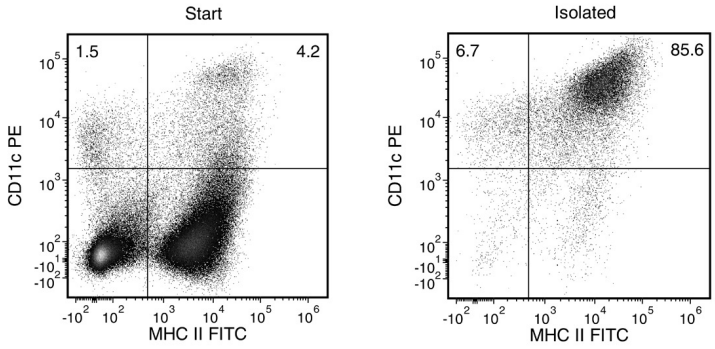
The EasySep™ Mouse CD11c Positive Selection Cocktail uses an anti-CD11c antibody clone that to our knowledge partially blocks most anti-CD11c antibody clones used to assess purity by flow cytometry. For purity assessment of CD11c+ cells by flow cytometry, use the following method:

- 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.