EasySep™ Mouse CD11c Positive Selection Kit II

Positive Selection

Catalog #18780

For labeling up to 2 x 10^9 cells

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Description

Isolate highly purified CD11c+ cells from mouse splenocytes, cultured bone marrow or other single-cell suspensions by positive selection.

- 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

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.
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.
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

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

This kit is designed to work with single-cell suspensions of mouse splenocytes. Incubate minced spleen in Spleen Dissociation Medium (Catalog #07915) for 30 minutes at room temperature (15 - 25°C). Dissociate spleen fragments into a smooth suspension by gently passing several times through a 18 gauge needle attached to a 3 cc Syringe (Catalog #28230). Pour the entire suspension through a pre-wetted 70 µm nylon mesh filter into a 50 mL conical screw-cap tube. Rinse the empty tube and mesh filter with 10 mL of PBS containing 2% fetal bovine serum (FBS) without EDTA (Catalog #07905) and add to the 50 mL conical tube. Centrifuge the 50 mL conical tube at 300 x g for 10 minutes and pour off the supernatant. Resuspend the cell pellet in ~0.5 mL of PBS containing 2% FBS (without EDTA) per spleen.

Add DNase I Solution (Catalog #07900) to a final concentration of 100 µg/ml and incubate for 10 minutes at room temperature. Count cells and resuspend in recommended medium (containing 1 mM EDTA) at 1 x 10^8 nucleated cells/mL.

Ammonium chloride treatment is not recommended when preparing the cells for separation.

For protocols with culture-expanded bone marrow-derived dendritic cells, please 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 Normal 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. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
4	Prepare Selection Cocktail in a tube. For each 1 mL of sample make 50 µL of cocktail (25 µl of Component A + 25 µL of Component B).	Mix equal volumes of Component A and Component B. Prepared cocktail is stable at 2 - 8°C for up to 2 weeks.	Mix equal volumes of Component A and Component B. Prepared 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™.	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 now ready for use	Isolated cells are now 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	
		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 Normal 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. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
4	Prepare Selection Cocktail in a tube. For each 1 mL of sample make 50 µL of cocktail (25 µl of Component A + 25 µL of Component B).	Mix equal volumes of Component A and Component B. Prepared cocktail is stable at 2 - 8°C for up to 2 weeks.	Mix equal volumes of Component A and Component B. Prepared 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™.	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 and 2 x 5-minute separations)	Steps 10 and 11 (total of 1 x 10 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 now ready for use	Isolated cells are now 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 and for the EasyEights™ 14 mL tube use a 10 mL serological pipette).

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 Normal Rat Serum to sample.	50 µL/mL of sample
3	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
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 2 weeks.
	Incubate.	RT for 5 minutes
5	Select protocol.	Mouse CD11c Positive Selection II 18780v2
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 now 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).

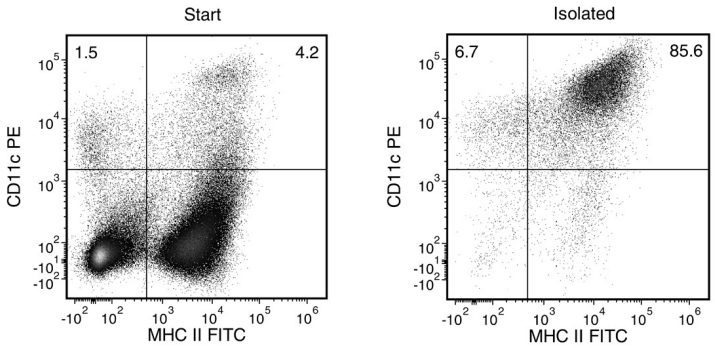
Notes and Tips

ASSESSING PURITY
For purity assessment by flow cytometry use fluorochrome-conjugated:
- Anti-Mouse CD11c Antibody, Clone N418 (Catalog #60002), and
- Anti-mouse MHC II antibody

Note: Other clones may be blocked and should be tested before use.

One of the following methods can also be used:
- 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.
- Use a fluorochrome-conjugated secondary antibody, such as a FITC-labeled sheep anti-mouse IgG.

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 example above, the final purities of the start and isolated fraction are 5.7% and 92.3%, respectively.

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