

EasySep™ Mouse Pan-DC Enrichment Kit II



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For processing 2 x 10⁹ cells

Catalog #19863
 #19863RF RoboSep™

Negative Selection

Document #1000006253 | Version 02

Description

Isolate untouched and highly purified dendritic cells (DCs), including conventional and plasmacytoid DCs, from mouse splenocytes by immunomagnetic negative selection. When using single-cell suspensions from other tissue types, this kit may require optimization.

- Fast, easy-to-use, and column-free
- Up to 65% purity
- Isolated cells are untouched
- Optimized for high yield
- Facilitates rapid flow sorting of DC subsets

This kit targets non-DCs for removal with biotinylated antibodies recognizing specific cell surface markers. Unwanted cells are labeled with biotinylated antibodies and magnetic particles, and separated without columns using an EasySep™ magnet. Desired cells are simply poured off into a new tube. Isolated cells are immediately available for downstream applications, such as flow cytometry, culture, or DNA/RNA extraction.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Mouse Pan-DC Enrichment Cocktail II	19863C	1 x 1 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. Includes an Fc receptor blocking antibody.
EasySep™ Streptavidin RapidSpheres™ 50001 ‡	50001	1 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.

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

‡ When using RoboSep™-S, contact us at techsupport@stemcell.com to request an additional vial of EasySep™ Streptavidin RapidSpheres™.

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Sample Preparation

For automated and standardized tissue processing, see STEMprep™ Tissue Dissociator (Catalog #100-2112) at www.stemcell.com/stemprep. For manual processing, follow the steps below.

SPLEEN

For maximum recovery, we recommend digesting the spleen at 37°C using Spleen Dissociation Medium (Catalog #07915). Refer to the Product Information Sheet for Spleen Dissociation Medium for more information.

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



Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% fetal bovine serum (FBS) and 1 mM EDTA. HBSS, Modified (Without Ca⁺⁺ and Mg⁺⁺; Catalog #37250) can be used in place of PBS. 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 Pan-DC Enrichment 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 0.25 - 2 mL	1 x 10 ⁸ cells/mL 0.5 - 8 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add Enrichment 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
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	40 µL/mL of sample	40 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	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	<ul style="list-style-type: none"> • 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
6	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring 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)

* 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 Pan-DC Enrichment 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 - 2 mL	1 x 10 ⁸ cells/mL 0.5 - 8 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add Enrichment 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
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add RapidSpheres™ to sample.	40 µL/mL of sample	40 µL/mL of sample
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
5	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	<ul style="list-style-type: none"> • 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
6	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 1 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

Table 3. RoboSep™ EasySep™ Mouse Pan-DC Enrichment Kit II 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 - 8 mL ‡	
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	
4	Select protocol.	Mouse Pan-DC Negative Selection II 19863	
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	
6	Load the carousel.	Follow on-screen prompts	
	Start the protocol.	Press the green “Run” button	
7	Unload the carousel when the run is complete.	Isolated cells are ready for use	

‡ When using RoboSep™-S, contact us at techsupport@stemcell.com to request an additional vial of EasySep™ Streptavidin RapidSpheres™.

Notes and Tips

ASSESSING PURITY

Conventional dendritic cells (cDCs) express high levels of CD11c, whereas plasmacytoid dendritic cells (pDCs) express lower levels of CD11c. PDCA-1 (BST-2) is specifically expressed by pDCs. cDCs are defined as Lin-CD11c+PDCA-1-, whereas pDCs are Lin-CD11c^{low}PDCA-1+.

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

- Anti-Mouse CD11c Antibody, Clone N418 (Catalog #60002), and
- Anti-mouse PDCA-1 antibody, and
- Anti-mouse lineage-specific antibodies (see below)

For lineage-specific antigen labeling, use the following fluorochrome-conjugated antibody clones:

- Anti-Mouse CD3e Antibody, Clone 145-2C11 (Catalog #60015), and
- Anti-Mouse CD19 Antibody, Clone 1D3 (Catalog #60112), and
- Anti-Mouse Ly-6G Antibody, Clone 1A8 (Catalog #60031), and
- Anti-Mouse F4/80 Antibody, Clone BM8 (Catalog #60027), and
- Anti-Mouse NK1.1 (CD161) Antibody, Clone PK136 (Catalog #60103), and
- Anti-Mouse TER119 Antibody, Clone TER-119 (Catalog #60033), and
- Anti-IgM antibody, clone 1B4B1

Data

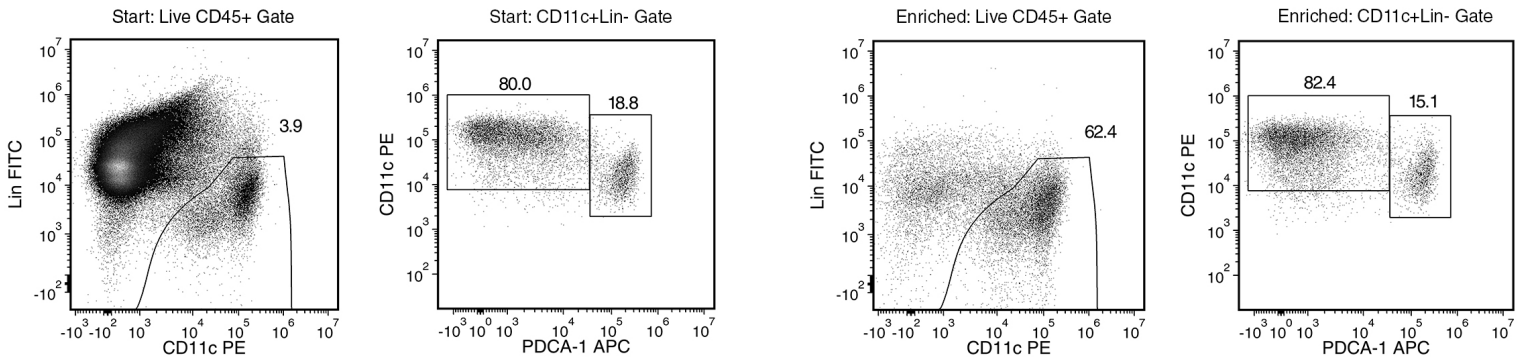


Figure 1. Enrichment of Pan-DCs from Mouse Splenocytes

Starting with mouse splenocytes, the dendritic cell content (CD11c+lineage-) of the isolated fraction is typically 57.3 ± 5.5% (mean ± SD using the purple EasySep™ magnet). In the above example, the purities of the start and final enriched fractions are 3.9% and 62.4%, respectively.

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