EasySep™ Mouse Pan-DC Enrichment Kit II

For processing 2 x 10^9 cells

Catalog #19863

Negative Selection

Document #10000006253 | Version 01

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
TOLL FREE PHONE 1 800 667 0322 • PHONE +1 604 877 0713
INFO@STEMCELL.COM • TECHSUPPORT@STEMCELL.COM
FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

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

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.**	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	• 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.

Document #10000006253 | Version 01

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.	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	• 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, wheras 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+.

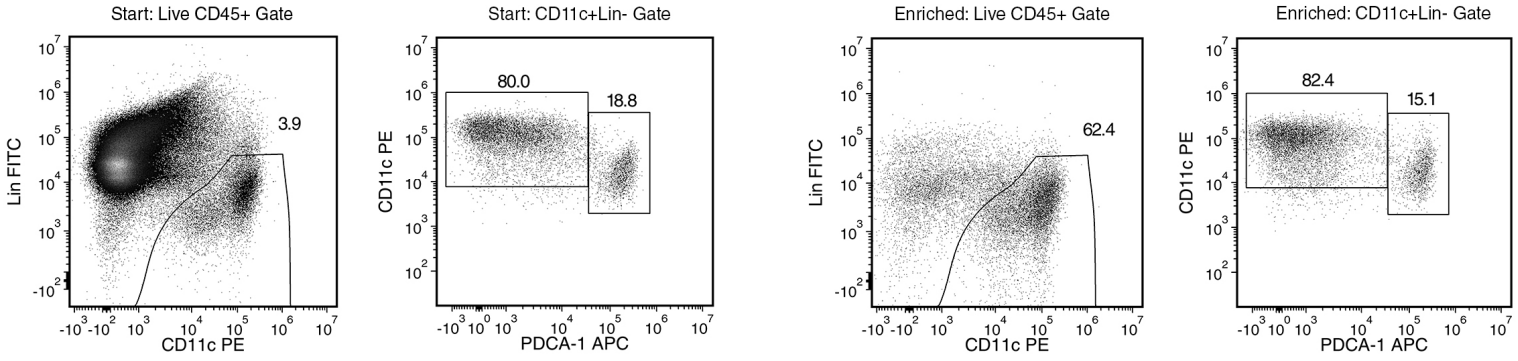
For purity assessment of pan-DCs by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- 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



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.