EasySep™ Mouse Pan-DC Enrichment Kit

Negative Selection

Catalog #19763

For processing 1 x 10^9 cells

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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 76% purity
- Isolated cells are untouched

This kit targets non-DCs for removal with biotinylated antibodies recognizing specific cell surface marker. 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	19763C	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™ Biotin Selection Cocktail	19153	2 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.
EasySep™ D Magnetic Particles	19250	4 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles in TBS.

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

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 (Document #29636) for the 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 Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Mouse Pan-DC Enrichment Kit 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. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Add Enrichment Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes	2 - 8°C for 15 minutes
3	Wash cells by topping up with recommended medium and centrifuge.	300 x g for 10 minutes	300 x g for 10 minutes
	Discard the supernatant and resuspend cells in the original volume with recommended medium.	0.25 - 2 mL	0.5 - 8 mL
4	Add Biotin Selection Cocktail to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	2 - 8°C for 10 minutes	2 - 8°C for 10 minutes
5	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add Magnetic Particles to sample.	75 µL/mL of sample	125 µL/mL of sample
	Mix and incubate.	2 - 8°C for 10 minutes	2 - 8°C for 10 minutes
7	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 2.5 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
8	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
9	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes
10	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.

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 2 for detailed instructions regarding the RoboSep™ procedure.

Table 2. RoboSep™ EasySep™ Mouse Pan-DC Enrichment Kit 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 - 6.5 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	**Add Enrichment Cocktail to sample.**	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes
3	**Wash cells by topping up with recommended medium and centrifuge.**	300 x g for 10 minutes
	Discard the supernatant and resuspend cells in the original volume with recommended medium.	0.5 - 6.5 mL
4	**Select protocol.**	• For volumes between 0.5 - ≤ 4 mL use: Mouse Pan-DC Negative Selection 19763-small volume • For volumes between > 4 - 6.5 mL use: Mouse Pan-DC Negative Selection 19763-large volume
5	**Vortex Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
6	**Load the carousel.**	Follow on-screen prompts NOTE: This protocol requires that **two** vials of EasySep™ D Magnetic Particles (Catalog #19250) be loaded onto the carousel for a single run.
	Start the protocol.	Press the green “Run” button
7	**Unload the carousel when the run is complete.**	Isolated cells are ready for use

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+.

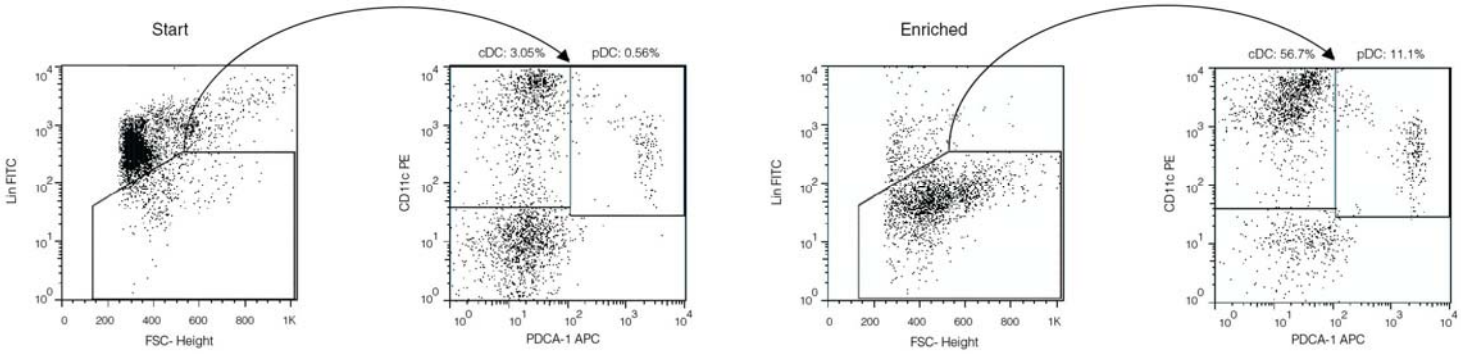
For purity assessment of pan-DCs by flow cytometry use the following fluorochrome-conjugated antibody clones:

- 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 of the isolated fraction is typically 65 ± 11%. In the above example, the purities of the start and final enriched fractions are 3.6% and 67.8%, respectively.