EasySep™ Mouse Plasmacytoid DC Isolation Kit

Negative Selection

Catalog #19764

For processing 2 x 10^9 cells

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Description

Isolate untouched and highly purified plasmacytoid dendritic cells (pDCs; PDCA-1+CD11c+) 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 94% purity
- Isolated cells are untouched

This kit targets non-pDCs 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 Plasmacytoid DC Isolation Cocktail	19764C	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™ D2 Magnetic Particles	19650	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

Disrupt spleen/lymph node in PBS or Hanks' Balanced Salt Solution containing 2% fetal bovine serum (FBS). Remove aggregates and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge at 300 x g for 10 minutes and resuspend at 1 x 10^8 nucleated cells/mL in recommended medium.

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% 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 Plasmacytoid DC Isolation 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 0.5 - 2 mL	1 x 10^8 cells/mL 0.5 - 8.5 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 Isolation 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 the 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 the cells in the original start volume.	0.5 - 2 mL	0.5 - 8.5 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.	37.5 µL/mL of sample	37.5 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 5 mL for samples ≤ 4 mL • Top up to 10 mL for samples > 4 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 new 5 mL tube	Use new 14 mL tube
9	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
10	Add Magnetic Particles to the new tube containing the enriched cell suspension.	7 µL	• 25 µL for samples ≤ 4 mL • 50 µL for samples > 4 mL
	Mix and incubate.	2 - 8°C for 5 minutes	2 - 8°C for 5 minutes
11	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
12	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™ Mouse Plasmacytoid DC Isolation 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 1 - 8 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Add Isolation Cocktail to sample.	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 15 minutes
3	Wash the cells by topping up with recommended medium and centrifuge.	300 x g for 10 minutes
	Discard the supernatant and resuspend the cells in the original start volume.	1 - 8 mL
4	Select protocol.	• For sample volumes ≤ 4 mL: Mouse Plasmacytoid DC Negative Selection 19764-small volume • For sample volumes > 4 mL: Mouse Plasmacytoid DC Negative Selection 19764-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™ D2 Magnetic Particles (Catalog #19650) 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. Remove the tube containing the isolated cells.	Isolated cells are ready for use

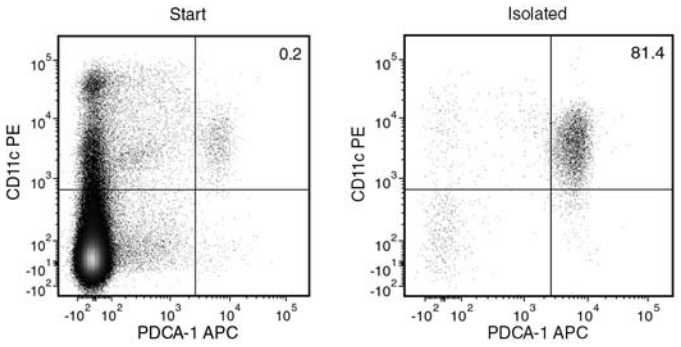
Notes and Tips

ASSESSING PURITY

For purity assessment of pDCs (PDCA-1+CD11c+) by flow cytometry use the following fluorochrome-conjugated antibody clone:

- Anti-Mouse CD11c Antibody, Clone N418 (Catalog #60002), and
- Anti-mouse PDCA-1 antibody

Data



Starting with mouse splenocytes, the pDC content (PDCA-1+CD11c+) of the enriched fraction typically ranges from 62 - 94%. In the above example, the purities of the start and final isolated fractions are 0.2% and 81.4%, respectively.