

# EasySep™ Mouse Plasmacytoid DC Isolation Kit

For processing  $2 \times 10^9$  cells

Catalog #19764

#19764RF RoboSep™

Negative Selection

Document #1000000925 | Version 01



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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

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

### 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 (Catalog #27215). Centrifuge at 300 x g for 10 minutes and resuspend at  $1 \times 10^8$  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 <sup>8</sup> cells/mL 0.5 - 2 mL	1 x 10 <sup>8</sup> cells/mL 0.5 - 8.5 mL
	Add sample to required tube.	5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	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. NOTE: Do not vortex cocktail.	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	<ul style="list-style-type: none"> <li>• Top up to 5 mL for samples ≤ 4 mL</li> <li>• Top up to 10 mL for samples &gt; 4 mL</li> </ul>
	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	<ul style="list-style-type: none"> <li>• 25 µL for samples ≤ 4 mL</li> <li>• 50 µL for samples &gt; 4 mL</li> </ul>
	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 #21000)	
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10 <sup>8</sup> cells/mL 1 - 8 mL	
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	
2	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	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.	<ul style="list-style-type: none"> <li>For sample volumes ≤ 4 mL: Mouse Plasmacytoid DC Negative Selection 19764-small volume</li> <li>For sample volumes &gt; 4 mL: Mouse Plasmacytoid DC Negative Selection 19764-large volume</li> </ul>	
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 <b>two</b> 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	

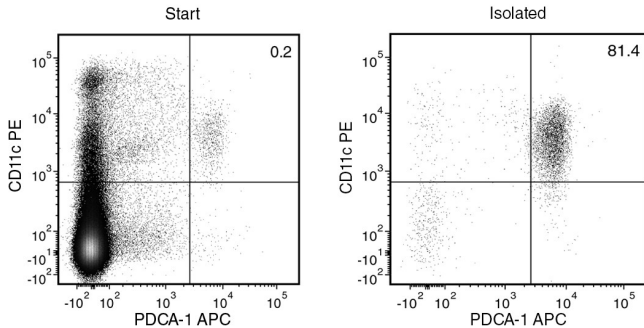
## Notes and Tips

### 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



**Figure 1. Isolation of Plasmacytoid Dendritic Cells from Mouse Splenocytes**

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.

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