EasySep™ Human Plasmacytoid DC Isolation Kit

For processing 2 x 10^9 cells

Catalog #17977

Negative Selection

Document #10000005859 | Version 00

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Description

Isolate untouched and highly purified plasmacytoid dendritic cells (pDCs) from fresh human peripheral blood mononuclear cells (PBMCs) or leukapheresis by immunomagnetic negative selection.

- Fast, easy-to-use, and column-free
- Up to 97% purity
- Untouched, viable cells

This kit targets non-pDCs including myeloid dendritic cells (mDCs) for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with 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™ Human Plasmacytoid DC Isolation Cocktail Component A	17977CA	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™ Human Plasmacytoid DC Isolation Cocktail Component B	17977CB	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™ Dextran RapidSpheres™ 50103	50103	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 water.
Anti-Human CD32 (Fc gamma RII) Blocker	14551C	2 x 0.8 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A combination of monoclonal antibodies in PBS.

PBS - phosphate-buffered saline

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

Sample Preparation

For available fresh and frozen samples, see www.stemcell.com/primarycells.

PERIPHERAL BLOOD

Prepare a PBMC suspension from whole peripheral blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube.

NOTE: Use of fresh whole blood is strongly recommended. Using day-old blood will result in reduced pDC purities and recoveries.

After preparation, resuspend cells at 5 x 10^7 cells/mL in recommended medium.

* SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. In all other regions SepMate™ is available for research use only (RUO).

LEUKAPHERESIS

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS). Centrifuge at 500 x g for 10 minutes at room temperature (15 - 25°C). If red blood cell (RBC) lysis is necessary, lyse with Ammonium Chloride Solution (Catalog #07800). If platelet removal is necessary, centrifuge at 120 x g for 10 minutes with the brake off. Remove the supernatant and resuspend the cells at 5 x 10^7 cells/mL in recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% fetal bovine serum (FBS) and 1 mM EDTA. 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™ Human 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.	5 x 10^7 cells/mL 0.25 - 2 mL	5 x 10^7 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 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add FcR Blocker to sample.‡	30 µL/mL of sample	30 µL/mL of sample
3	Add Plasmacytoid DC Isolation Cocktail Component A to sample.	50 µL/mL of sample	50 µL/mL of sample
4	Add Plasmacytoid DC Isolation Cocktail Component B to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 1 minute	RT for 1 minute
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 3 minutes	RT for 3 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 1 minute	RT for 1 minute
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)
‡ Addition of Anti-Human CD32 (Fc gamma RII) Blocker may prevent downstream attempts at cross-linking CD32 molecules to trigger signaling through these receptors. The FcR Blocker may be omitted if desired.
* 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.

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Table 2. EasySep™ Human Plasmacytoid DC Isolation Kit Protocol

		EASYSEP™ MAGNETS		
		EasyEights™ (Catalog #18103)		
STEP	INSTRUCTIONS	5 mL tube	14 mL tube	Easy 50 (Catalog #18002)
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 0.5 - 2 mL	5 x 10^7 cells/mL 0.5 - 8.5 mL	5 x 10^7 cells/mL 5 - 35 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)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add FcR Blocker to sample.‡	30 µL/mL of sample	30 µL/mL of sample	30 µL/mL of sample
3	Add Plasmacytoid DC Isolation Cocktail Component A to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
4	Add Plasmacytoid DC Isolation Cocktail Component B to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
5	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
6	Add RapidSpheres™ to sample and mix.	100 µL/mL of sample	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 1 minute	RT for 1 minute	RT for 5 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	• Top up to 25 mL for samples ≤ 20 mL • Top up to 50 mL for samples > 20 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 10 minutes	RT for 10 minutes
8	Carefully pipette*** (do not pour) the supernatant into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 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 10 minutes	RT for 10 minutes	RT for 10 minutes
10	Carefully pipette*** (do not pour) the supernatant into a new tube.	Isolated cells are ready for use	Isolated cells are ready for use	Use a new 50 mL tube
11	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a third separation.	---	---	RT for 5 minutes
12	Carefully pipette*** (do not pour) the supernatant into a new tube.	---	---	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
‡ Addition of Anti-Human CD32 (Fc gamma RII) Blocker may prevent downstream attempts at cross-linking CD32 molecules to trigger signaling through these receptors. The FcR Blocker may be omitted if desired.
*** Collect the entire enriched cell suspension, 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™ Human Plasmacytoid DC Isolation Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL 0.5 - 8 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add FcR Blocker to sample.‡	30 µL/mL of sample
3	Select protocol.	Human Plasmacytoid DC Negative Selection 17977
4	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
5	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
6	Unload the carousel when the run is complete.	Isolated cells are ready for use

‡ Addition of Anti-Human CD32 (Fc gamma RII) Blocker may prevent downstream attempts at cross-linking CD32 molecules to trigger signaling through these receptors. The FcR Blocker may be omitted if desired.

Notes and Tips

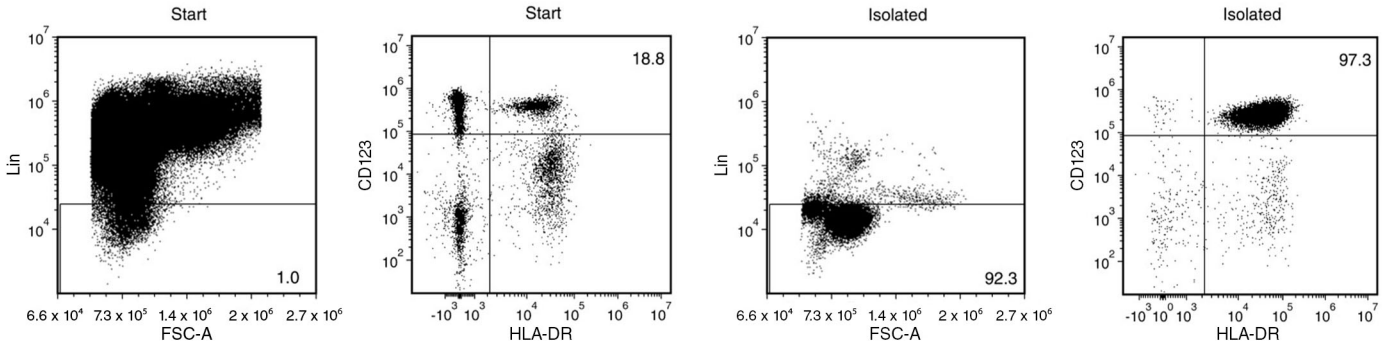
ASSESSING PURITY

pDCs are described as Lineage (CD3, CD14, CD16, CD19, CD20, CD34, CD56)-negative, HLA-DR positive, and CD123 (IL-3Rα)-positive. For purity assessment of pDCs by flow cytometry, use the following fluorochrome-conjugated antibodies:

- Anti-Human CD3 Antibody, Clone SK7 (Catalog #60127), and
- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004), and
- Anti-Human CD16 Antibody, Clone 3G8 (Catalog #60041), and
- Anti-Human CD19 Antibody, Clone HIB19 (Catalog #60005), and
- Anti-Human CD20 Antibody, Clone 2H7 (Catalog #60008), and
- Anti-Human CD34 Antibody, Clone 581 (Catalog #60013), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018), and
- Anti-Human CD56 (NCAM) Antibody, Clone HCD56 (Catalog #60021), and
- Anti-Human HLA-DR Antibody, Clone LN3 (Catalog #60164), and
- Anti-Human CD123 (IL-3Rα) Antibody, Clone 6H6 (Catalog #60110)

NOTE: Include a viability dye if necessary (e.g. Propidium Iodide [Catalog #75002] or 7-AAD [7- Aminoactinomycin D; Catalog #75001]).

Data



Starting with leukapheresis samples, the pDC content (Lin-HLA-DR+CD123+) of the isolated fraction is typically 90 ± 5.3% (mean ± SD using the silver “Big Easy” EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 0.2% and 89.8%, respectively.