EasySep™ Human Plasmacytoid DC Enrichment Kit

Negative Selection

Catalog #19062

For processing 2 x 10^9 cells

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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

Document #29149 | Version 2_1_2

Description

Isolate untouched and highly purified plasmacytoid dendritic cells (pDCs) from fresh human peripheral blood mononuclear cells (PBMCs) 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 Enrichment Cocktail	19062C	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.
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.
Anti-Human CD32 (Fc gamma RII) Blocker for negative selection	14551C	1 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; TBS - TRIS-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).

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 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.**	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. Corning Catalog #352058)	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	**Add FcR Blocker to sample.‡**	30 µL/mL of sample	30 µL/mL of sample
3	**Add Enrichment Cocktail to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 30 minutes	RT for 30 minutes
4	**Vortex Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	30 seconds	30 seconds
5	**Add Magnetic Particles to sample.**	200 µL/mL of sample	200 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
6	**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 3.5 mL for samples ≤ 2 mL • Top to 6.5 mL for samples > 2 - 5 mL • Top up to 10 mL for samples > 5 mL
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes
7	**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
8	**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
9	**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. Anti-Human CD32 (Fc gamma RII) Blocker may be omitted if necessary.
* 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.

Table 2. EasySep™ Human Plasmacytoid DC Enrichment Kit Protocol

		EASYSEP™ MAGNETS
STEP	INSTRUCTIONS	**Easy 50 (Catalog #18002)** 
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10^7 cells/mL Up to 35 mL
	Add sample to required tube.	50 mL conical tube (e.g. Corning Catalog #352070)
2	Add FcR Blocker to sample.‡	30 µL/mL of sample
3	Add Enrichment Cocktail to sample.	50 µL/mL of sample
	Mix and incubate.	RT for 30 minutes
4	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds
5	Add Magnetic Particles to sample.	250 µL/mL of sample
	Mix and incubate.	RT for 10 minutes
6	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	• Top up to 10 mL for samples < 5 mL • Top up to 20 mL for samples ≥ 5 - 10 mL • Top up to 30 mL for samples > 10 - 15 mL • Top up to 40 mL for samples > 15 - 20 mL • Top up to 50 mL for samples > 20
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes
7	Carefully pipette** (do not pour) the enriched cell suspension into a new tube.	Use a new 50 mL tube
8	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
9	Carefully pipette** (do not pour) the enriched cell suspension 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. Anti-Human CD32 (Fc gamma RII) Blocker may be omitted if necessary.
** Collect the entire supernatant, all at once, into a single pipette.

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 Enrichment Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #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 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)
2	Add FcR Blocker to sample.‡	30 µL/mL of sample
3	Select protocol.	• Human pDC Enrichment 19062-small volume (0.5 - 2.0 mL) • Human pDC Enrichment 19062-large volume (2.1 - 5.0 mL) • Human pDC Enrichment 19062-mega volume (5.1 - 8.0 mL)
4	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds
5	Load the carousel.	Follow on-screen prompts NOTE: This protocol requires loading **two** vials of EasySep™ D Magnetic Particles onto the carousel for a single run; one in the ▲ (triangle) slot and one in the ● (circle) slot.
	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. Anti-Human CD32 (Fc gamma RII) Blocker may be omitted if necessary.

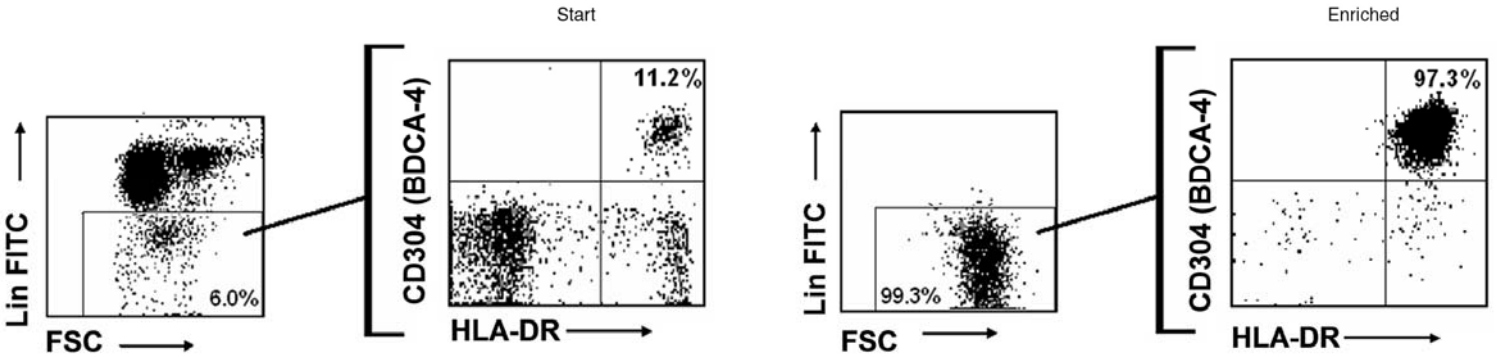
Notes and Tips

ASSESSING PURITY

pDCs are described as Lineage (CD3, CD14, CD16, CD19, CD20, CD34, CD56)-negative, HLA-DR positive, and CD304 (BDCA-4)-positive. For purity assessment of pDCs by flow cytometry use the following fluorochrome-conjugated antibodies:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), 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 CD56 (NCAM) Antibody, Clone HCD56 (Catalog #60021), and
- Anti-Human HLA-DR Antibody, Clone LN3 (Catalog #60164), and
- Anti-human CD304 (BDCA-4) antibody

Data



Starting with PBMCs containing 0.2 - 0.9% pDCs, the pDC content (Lin-/HLA-DR+/BDCA-4+) of the enriched fraction typically ranges from 87 - 97%. In the above example, the purities of the start and final enriched fractions are 0.67% and 96.6%, respectively.