EasySep™ Human Pan-DC Pre-Enrichment Kit

Negative Selection

Catalog #19251

For processing 1 x 10^9 cells

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Description

Isolate untouched pre-enriched dendritic cells (including myeloid and plasmacytoid dendritic cells; DCs) from fresh human peripheral blood mononuclear cells (PBMCs) by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 80% purity
- Untouched, viable cells

This kit targets non-DCs 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 Pan-DC Pre-Enrichment Cocktail	19251C.1	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	2 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	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 without the need for careful sample layering, use the SepMate™-15 (Catalog #15415) or SepMate™-50 (Catalog #15450) cell isolation tube.

If using previously frozen PBMCs, thaw up to 5 x 10^8 cells in 50 mL of PBS containing 20% fetal bovine serum (FBS) and 1 mM EDTA. Centrifuge at 200 x g for 10 minutes. Carefully remove and discard the supernatant and resuspend the cells in recommended medium. Centrifuge at 200 x g for 10 minutes and carefully remove and discard the supernatant. DNase I treatment is not recommended when preparing the cells for separation.

After preparation, resuspend 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% 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 Pan- DC Pre-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 - 8.5 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 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.	30 seconds	30 seconds
5	Add Magnetic Particles to sample.	100 µL/mL of sample	100 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 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
7	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the enriched cell suspension into a new tube.	Use a new 5 mL tube	Use a new 14 mL tube
OPTIONAL ADDITIONAL SEPARATION Note: This will increase purity but may decrease recovery. Use of this protocol will require additional Magnetic Particles. Please contact Technical Support to request more.		---	---
8	Add Magnetic Particles to sample.	100 µL/mL of sample	100 µL/mL of sample
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
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)

‡ Addition of Anti-Human CD32 (Fc gamma RII) Blocker is optional but may improve product performance by preventing non-specific depletion of DCs. Use of the Anti-Human CD32 (Fc gamma RII) Blocker may prevent subsequent attempts at cross-linking CD32 molecules on the surface of enriched cells to trigger signaling through these receptors. It may therefore be desirable to not add the Anti-Human CD32 (Fc gamma RII) Blocker to the cell suspension for such studies.

* 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 Pan- DC Pre-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 1 - 35 mL
	Add sample to required tube.	50 mL conical tube (e.g. Corning Catalog #352070)
2	Add 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.	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 mL
	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 round of 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 is optional but may improve product performance by preventing non-specific depletion of DCs. Use of the Anti-Human CD32 (Fc gamma RII) Blocker may prevent subsequent attempts at cross-linking CD32 molecules on the surface of enriched cells to trigger signaling through these receptors. It may therefore be desirable to not add the Anti-Human CD32 (Fc gamma RII) Blocker to the cell suspension for such studies.
** 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 Pan-DC Pre-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 - 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 Blocker to sample.	30 µL/mL of sample
3	Select protocol.	Human panDC pre-Enrichment Negative Selection 19251-high recovery
4	Vortex Magnetic Particles .	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

Notes and Tips

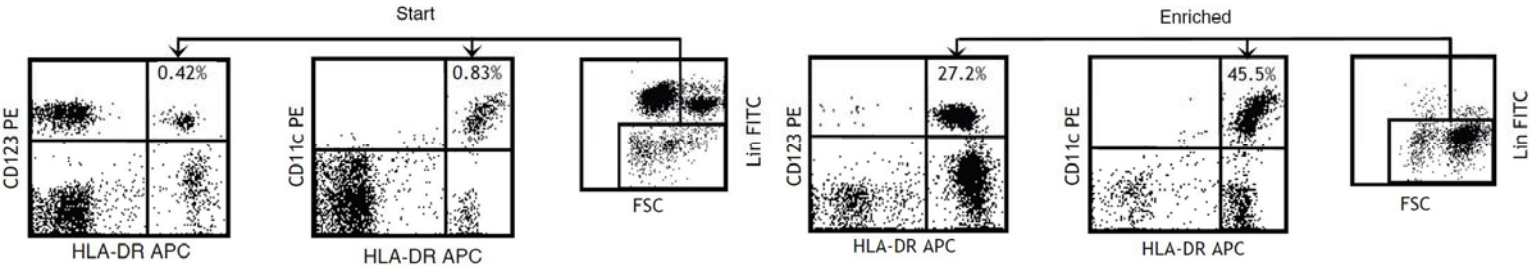
ASSESSING PURITY

For purity assessment of pan-DCs by flow cytometry use an appropriate combination of fluorochrome-conjugated:

- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004), and
- Anti-Human CD16 Antibody, Clone 3G8 (Catalog #60041), and
- Anti-Human CD20 Antibody, Clone 2H7 (Catalog #60008), and
- Anti-Human CD56 (NCAM) Antibody, Clone HCD56 (Catalog #60021), and
- Anti-human HLA-DR antibody, and
- Anti-human TCR alpha/beta antibody, and
- Anti-Human CD123 (IL-3Ra) Antibody, Clone 6H6 (Catalog #60110), or anti-human CD11c antibody

DCs are described as either Lineage- (CD14, CD16, CD20, CD56, TCRab), HLA-DR+CD123+ or Lineage- (CD14, CD16, CD20, CD56, TCRab), HLA-DR+CD11c+.

Data



Starting with peripheral blood mononuclear cells, the dendritic cell content (Lin-/HLA-DR+/CD123+ or CD11c+) of the enriched fraction typically ranges from 40 - 80%. In the above example, the final purities of the start and enriched fractions are 1.25% and 72.7%, respectively.