

EasySep™ Human Pan-DC Pre-Enrichment Kit

For processing 1×10^9 cells

Catalog #19251

Negative Selection

Document #1000000880 | Version 01



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Description

Isolate untouched pre-enriched dendritic cells (including myeloid and plasmacytoid dendritic cells; DCs) from fresh or previously frozen 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 Component A	19251C.2	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™ Human DC Enrichment Cocktail Component B	19060C	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, 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

‡ When using the Easy 50 EasySep™ Magnet, contact us at techsupport@stemcell.com to request three additional vials of EasySep™ D Magnetic Particles.

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 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.

If using previously frozen PBMCs, thaw up to 5×10^8 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 5×10^7 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% 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


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. 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 Pan-DC Pre-Enrichment Cocktail Component A to sample.	50 µL/mL of sample	50 µL/mL of sample
4	Add DC Enrichment Cocktail Component B to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	RT for 30 minutes	RT for 30 minutes
5	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
6	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
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"> • 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
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
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 additional particles.		---	---
9	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
10	Add Magnetic Particles to sample.	100 µL	100 µL
	Mix and incubate.	RT for 10 minutes	RT for 10 minutes
11	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for another separation.	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.	Use a new 5 mL tube	Use a new 14 mL tube
13	Repeat steps as indicated.	Steps 11 and 12 (for a total of 2 x 5-minute separations) Isolated cells are ready for use	Steps 11 and 12 (for a total of 2 x 5-minute separations) 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 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 Pan-DC Pre-Enrichment Kit Protocol

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 (30 x 115 mm) conical tube (e.g. Catalog #38010)	
2	Add FcR Blocker to sample. §	30 µL/mL of sample	
3	Add Pan-DC Pre-Enrichment Cocktail Component A to sample.	50 µL/mL of sample	
4	Add DC Enrichment Cocktail Component B to sample.	50 µL/mL of sample	
	Mix and incubate.	RT for 30 minutes	
5	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds	
6	Add Magnetic Particles to sample.	250 µL/mL of sample	
	Mix and incubate.	RT for 10 minutes	
7	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<ul style="list-style-type: none"> • 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	
8	Carefully pipette** (do not pour) the enriched cell suspension into a new 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 round of separation.	RT for 5 minutes	
10	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)

‡ When using the Easy 50 EasySep™ Magnet, contact us at techsupport@stemcell.com to request three additional vials of EasySep™ D Magnetic Particles.


§ 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 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 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 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)	
2	Add FcR Blocker to sample. [§]	30 µL/mL of sample	
3	Add DC Enrichment Cocktail Component B to sample.	50 µL/mL of sample	
4	Select protocol.	Human panDC pre-Enrichment Negative Selection 19251-high recovery	
5	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds	
6	Load the carousel.	Follow on-screen prompts	
	Start the protocol.	Press the green “Run” button	
7	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 necessary.

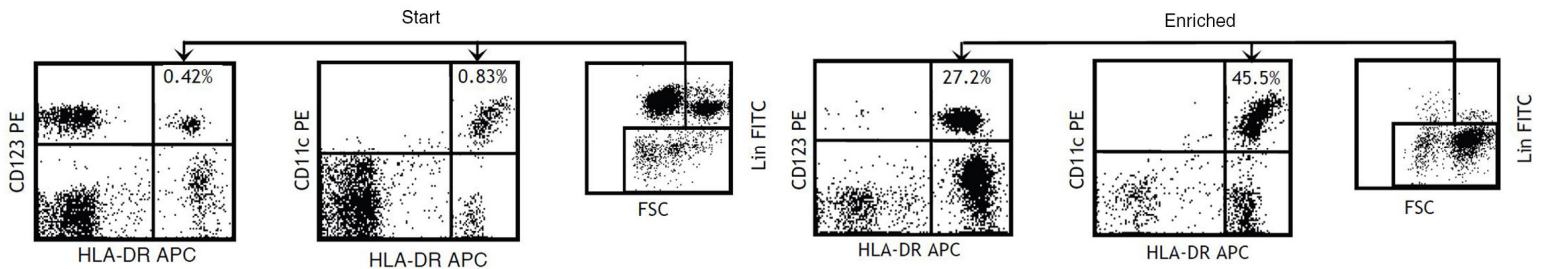
Notes and Tips

ASSESSING PURITY

DCs are described as either Lineage- (CD14, CD16, CD20, CD56, TCRab), HLA-DR+CD123+ or Lineage- (CD14, CD16, CD20, CD56, TCRab), HLA-DR+CD11c+. For purity assessment of pan-DCs by flow cytometry, use the following fluorochrome-conjugated antibodies:

- 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, Clone LN3 (Catalog #60164), and
- Anti-human TCR alpha/beta antibody, and
- Anti-Human CD123 (IL-3Ra) Antibody, Clone 6H6 (Catalog #60110), or anti-human CD11c antibody

Data



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

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