EasySep™ Human Myeloid DC Enrichment Kit

For processing 2 x 10^9 cells

Catalog #19061

Negative Selection

Document #10000000857 | Version 01

STEMCELL™ TECHNOLOGIES
Scientists Helping Scientists™ | WWW.STEMCELL.COM
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

Description

Isolate untouched and highly purified myeloid dendritic cells (mDCs) from fresh human peripheral blood mononuclear cells (PBMCs), buffy coat, or lysed leukapheresis samples by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 90% purity
- Untouched, viable cells

This kit targets non-mDCs including plasmacytoid dendritic cells (pDCs) 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 Myeloid DC Enrichment Cocktail Component A	19061C.1	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 DC Enrichment Cocktail Component B	19060C	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™ D Magnetic Particles	19250	5 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 mDC 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

1. Add an equal volume of Ammonium Chloride Solution (Catalog #07800) to the Leukopak.
   NOTE: If working with large volumes (> 150 mL), concentrate the Leukopak first by centrifuging at 500 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original Leukopak volume with recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of recommended medium and add 30 mL of Ammonium Chloride Solution). For small volumes (≤ 150 mL), add Ammonium Chloride Solution directly to the Leukopak.
2. Incubate for 15 minutes on ice.
3. Centrifuge at 500 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 150 x g for 10 minutes at room temperature with the brake off. Carefully remove the supernatant.
5. Repeat step 4 one or more times until most of the platelets have been removed (indicated by a clear supernatant).
6. 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 Table 1 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human Myeloid 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 0.5 - 2 mL	5 x 10^7 cells/mL 1 - 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. NOTE: Blocker addition is optional but highly recommended.	15 µL/mL of sample	15 µL/mL of sample
3	Add Myeloid DC 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	• 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 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
9	Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate.	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)

* 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™ Human Myeloid DC Enrichment 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 - 8.5 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.** **NOTE: Blocker addition is optional but highly recommended.**	15 µL/mL of sample
3	**Add DC Enrichment Cocktail Component B to sample.**	50 µL/mL of sample
4	**Select protocol.**	Human mDC Negative Selection 19061
5	**Vortex Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
6	**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
7	**Unload the carousel when the run is complete.**	Isolated cells are ready for use

‡ If starting with less than 1 mL, contact us at techsupport@stemcell.com to request an additional vial of EasySep™ D Magnetic Particles.

Notes and Tips

ASSESSING PURITY

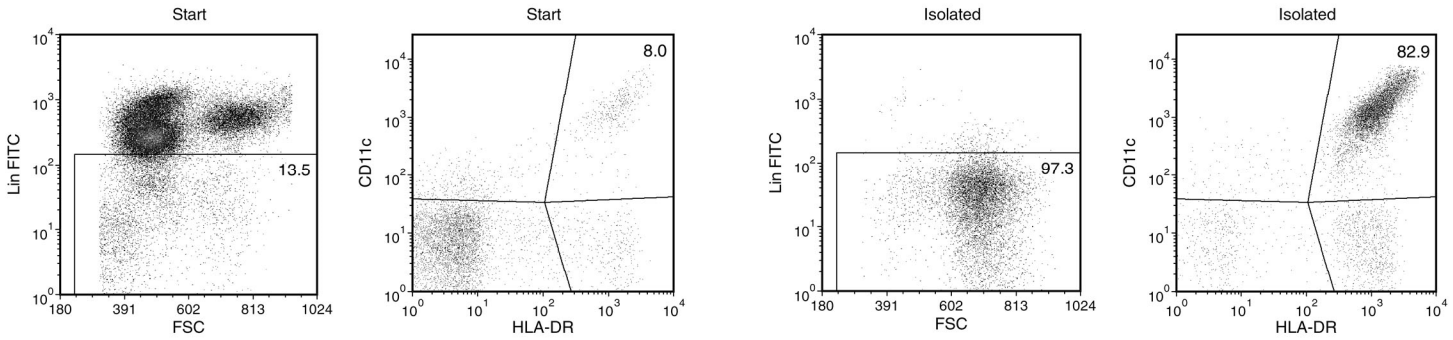
mDCs are described as Lineage (CD3, CD14, CD19, CD20, CD34, CD56)-negative, HLA-DR-positive and CD11c-positive. For purity assessment of mDCs by flow cytometry use the following fluorochrome-conjugated antibodies:

- Anti-human CD11c antibody, and
- Anti-Human HLA-DR Antibody, Clone LN3 (Catalog #60164), and
- Anti-human lineage-specific antibodies (see below)

For lineage-specific antigen labeling 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 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)

Data



Starting with 0.6 - 1.8% mDCs in fresh peripheral blood nucleated cells, the mDC content of the enriched fraction typically ranges from 50 - 90%* purity based on the mDC phenotype of Lineage (CD3, CD14, CD19, CD20, CD34, CD56)-negative, HLA-DR-positive, and CD11c-positive. In the above example, the purities of the start and final enriched fractions are 1.1% and 80.7%, respectively.

*If the mDC content of the starting sample is < 1.25%, the mDC content of the enriched fraction may be < 80%.