

EasySep™ Human Monocyte Isolation Kit

Negative Selection
Catalog #19359

For processing 1 x 10⁹ cells



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Description

Isolate untouched and highly purified CD14+CD16- monocytes from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or washed leukapheresis samples in as little as 12.5 minutes by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 94% purity with high recovery
- Untouched, viable cells

This kit targets non-monocytes, CD16+ monocytes, and platelets 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 Monocyte Isolation Cocktail	19359C	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. Includes an Fc receptor blocking antibody.
EasySep™ Human Platelet Removal Cocktail	19369C	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 for Human Monocytes	19550	1 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.

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

The presence of EDTA is important for the performance of this kit. Collect blood using K2EDTA or K3EDTA as an anticoagulant. If an anticoagulant other than EDTA is used, EDTA must be added to the whole blood sample to a final concentration of 3 mM.

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, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes prior to labeling and separation. Filter aggregated suspensions through a 40 µm Cell Strainer (Catalog #27305) for optimal results.

After preparation, resuspend cells at 5 x 10⁷ 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 (LEUKO PAK)

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium and centrifuging at 120 x g for 10 minutes at room temperature (15 - 25°C). Repeat the wash. If red blood cell (RBC) lysis is desired, lyse with Ammonium Chloride Solution (Catalog #07800) before the first wash step. Remove the supernatant and resuspend the cells at 5 x 10⁷ 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 Monocyte 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 0.5 - 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 Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Add Platelet Removal Cocktail to sample. NOTE: Optional, see Notes and Tips on page 4.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.*	RT for 5 minutes	RT for 5 minutes
3	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
4	Add Magnetic Particles to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.*	RT for 5 minutes	RT for 5 minutes
5	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 2.5 minutes	RT for 2.5 minutes
6	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)

* If incubation at 2 - 8°C is desired, increase the cocktail incubation time to 10 minutes. No increase in the particle incubation time is required.

** 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 Monocyte Isolation Kit Protocol

STEP	INSTRUCTIONS	EASYSEP™ MAGNETS			
		 EasyPlate™ (Catalog #18102)	 EasyEights™ (Catalog #18103) 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 0.05 - 0.2 mL	5 x 10 ⁷ cells/mL 0.5 - 2 mL	5 x 10 ⁷ cells/mL 1 - 8.5 mL	5 x 10 ⁷ cells/mL 1 - 40 mL
	Add sample to required tube (or plate if using the EasyPlate™ EasySep™ Magnet).	Round-bottom, non-tissue culture-treated 96-well plate (e.g. Corning Catalog #3788)	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)	50 mL conical tube (e.g. Corning Catalog #352070)
2	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Add Platelet Removal Cocktail to sample. NOTE: Optional, see Notes and Tips on page 4.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.*	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
3	Vortex Magnetic Particles. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds	30 seconds
4	Add Magnetic Particles to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.*	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
5	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 0.25 mL	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 	<ul style="list-style-type: none"> • Top up to 25 mL for samples ≤ 10 mL • Top up to 50 mL for samples > 10 mL
	Place the tube or plate (without lid) into the magnet and incubate.	RT for 10 minutes	RT for 2.5 minutes	RT for 2.5 minutes	RT for 10 minutes
6	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube or plate.	Isolated cells are ready for use	Use a new 5 mL tube	Use a new 14 mL tube	Isolated cells are ready for use
7	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 2.5 minutes	RT for 2.5 minutes	---
8	Carefully pipette*** (do not pour) 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)


* If incubation at 2 - 8°C is desired, increase the cocktail incubation time to 10 minutes. No increase in the particle incubation time is required.

*** Collect the entire supernatant, all at once, into a single pipette (e.g. for the EasyEights™ 5 mL tube use a 2 mL serological pipette and for the EasyEights™ 14 mL tube use a 10 mL serological 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 Monocyte Isolation 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 - 8.5 mL	
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)	
2	Add Platelet Removal Cocktail to sample. NOTE: Optional, see Notes and Tips on page 4.	50 µL/mL of sample	
3	Select protocol.	Human Monocyte Isolation 19359	
4	Vortex Magnetic Particles. 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	

Notes and Tips

PLATELET REMOVAL

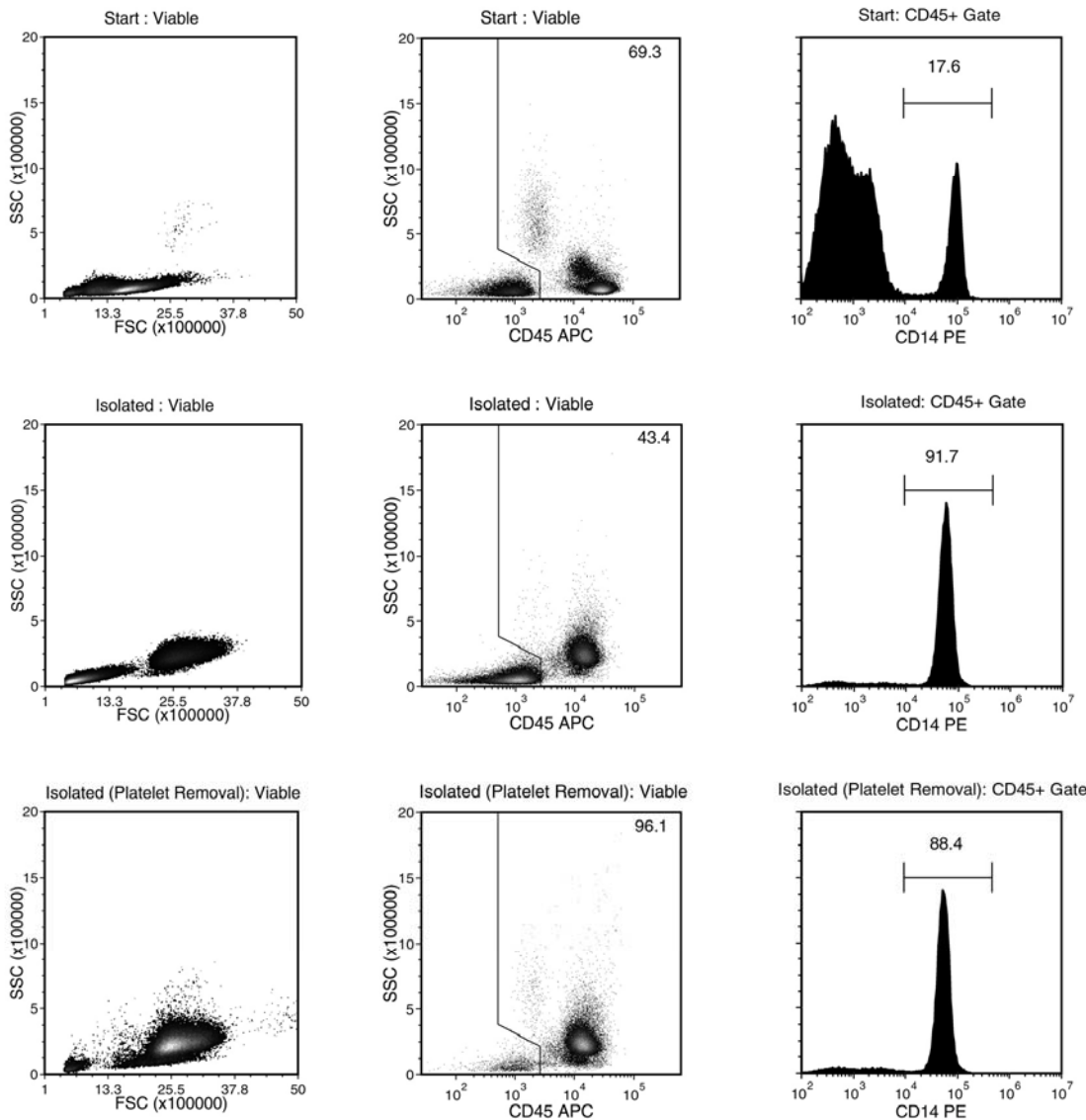
EasySep™ Human Platelet Removal Cocktail performs optimally when used with PBMCs prepared from human whole peripheral blood when following the Sample Preparation procedure for generation of PBMCs. The use of EasySep™ Human Platelet Removal Cocktail with leukapheresis samples or previously frozen PBMCs may result in lower recovery of monocytes.

ASSESSING PURITY

For purity assessment of monocytes (CD14+CD45+) by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004) and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)

Data



Starting with PBMCs prepared from human whole peripheral blood, the monocyte cell content (CD14+CD45+) of the isolated fraction obtained without (middle plots) or with EasySep™ Human Platelet Removal Cocktail (bottom plots) is typically $89.7 \pm 3.4\%$ and $87.3 \pm 4.5\%$, respectively (gated on CD45, mean \pm SD for the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions obtained without (middle plots) or with the EasySep™ Human Platelet Removal Cocktail (bottom plots) are 17.6%, 91.7%, and 88.4%, respectively (gated on CD45) and 12.2%, 39.8%, and 85.0% (not gated on CD45).

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