EasySep™ Human Monocyte Isolation Kit

Negative Selection

Catalog #19359

For labeling up to 1 x 10e9 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 apheresis 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.
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

WHOLE 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 peripheral blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation without need for careful sample layering, use the SepMate™-15 (Catalog #15415) or SepMate™-50 (Catalog #15450) cell isolation tube. Ensure that recommended medium containing EDTA is used for all sample preparation steps. For optimal platelet removal, 2 wash steps should be performed at 120 x g following density gradient centrifugation.

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

After preparation resuspend cells at 5 x 10e7 cells/mL in recommended medium.

PERIPHERAL BLOOD APHERESIS (LEUKOPAK)

Wash the peripheral blood apheresis sample by adding an equivalent volume of recommended medium and centrifuging at 120 x g for 10 minutes at room temperature. Repeat the wash. If red blood cell (RBC) lysis is necessary, an Ammonium Chloride (Catalog #07800) lysis may be done before the first wash step. Remove supernatant and resuspend the cells at 5 x 10e7 cells/mL in recommended medium.

Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or phosphate-buffered saline (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 10e7 cells/mL 0.5 - 2 mL	5 x 10e7 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.**	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	• 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 off the enriched cell suspension** into a new tube.**	Isolated cells are now ready for use	Isolated cells are now ready for use

RT - room temperature (15 - 25°C)
* If incubation at 4˚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

		EASYSEP™ MAGNETS			
STEP	INSTRUCTIONS	EasyPlate™ (Catalog #18102)	EasyEights™ (Catalog #18103) 5 mL tube	EasyEights™ (Catalog #18103) 14 mL tube	Easy 50 (Catalog #18002)
1	Prepare sample at the indicated cell concentration within the volume range.	5 x 10e7 cells/mL 0.05 - 0.2 mL	5 x 10e7 cells/mL 0.5 - 2 mL	5 x 10e7 cells/mL 1 - 8.5 mL	5 x 10e7 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. Costar Catalog #3788 or Corning® Catalog #351177)	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.	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	• Top up to 5 mL for samples < 2 mL • Top up to 10 mL for samples ≥ 2 mL	• 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 now ready for use	Use a new 5 mL tube	Use a new 14 mL tube	Isolated cells are now 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 now ready for use	Isolated cells are now ready for use	---

RT - room temperature (15 - 25°C)
* If incubation at 4˚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

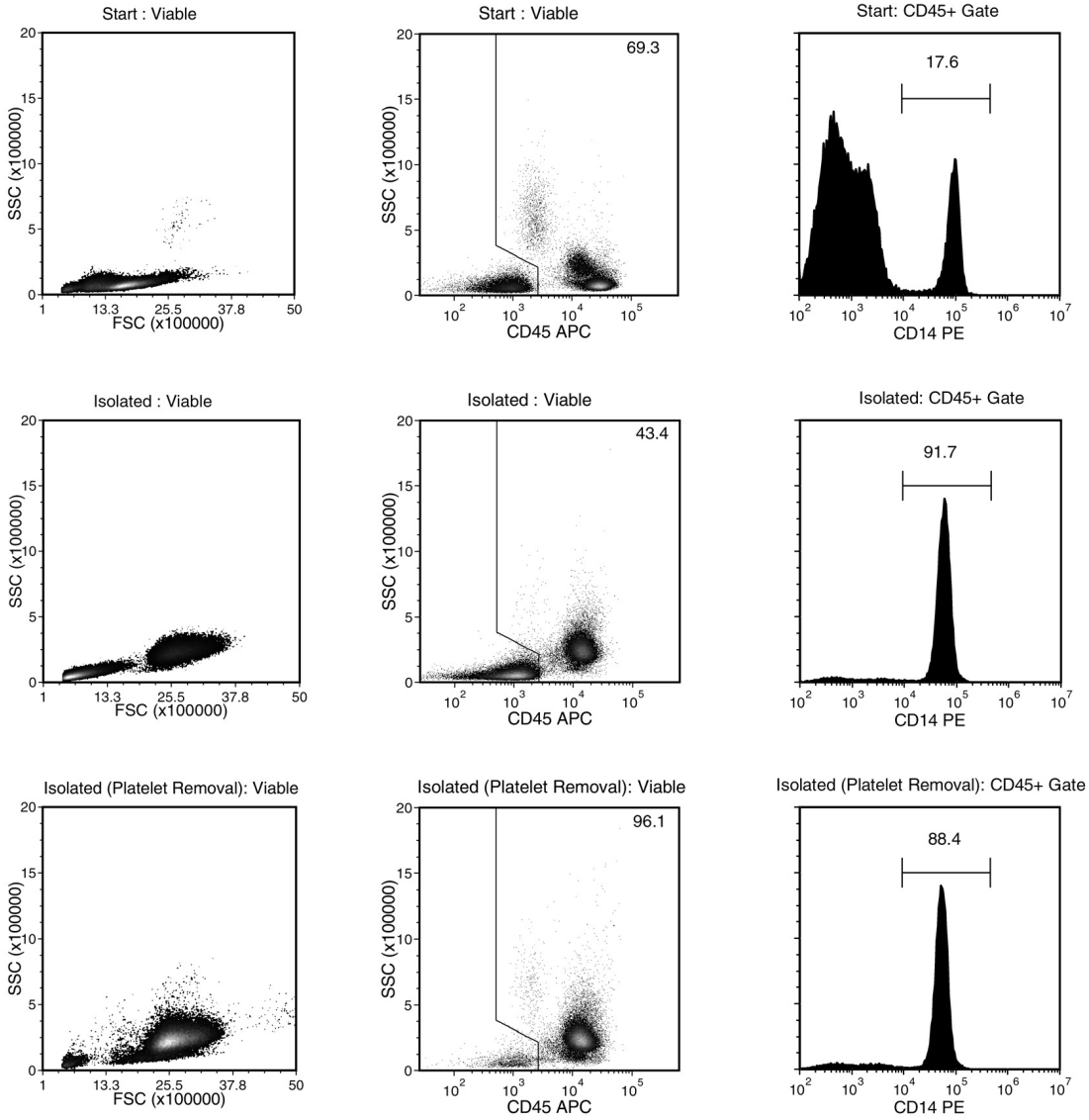
STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000) 
1	**Prepare sample at the indicated cell concentration within the volume range.**	5 x 10e7 cells/mL 0.5 - 8.5 mL
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning® Catalog #352057)
2	**Add Platelet Removal 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.**	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. Remove the tube containing the isolated cells.**	Isolated cells are now 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 leukopak samples or previously frozen PBMCs may result in lower recovery of monocytes.

ASSESSING PURITY
For purity assessment of monocytes (CD45+CD14+) by flow cytometry use fluorochrome-conjugated:
- 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 ± 3.4% and 87.3 ± 4.5%, respectively (gated on CD45, mean ± 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).