EasySep™ Human Monocyte Isolation Kit

For processing 1 x 10^9 cells

Catalog #19359
Catalog #19359RF RoboSep™

Negative Selection

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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 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 (e.g. Human Whole Peripheral Blood*, Catalog #70507) by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07811). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD** (Catalog #85450/85415) cell isolation tube, or source fresh PBMCs (e.g. Human Peripheral Blood Mononuclear Cells, Fresh*, Catalog #200-0077).

If using previously frozen PBMCs (e.g. Human Peripheral Blood Mononuclear Cells, Frozen*, Catalog #70025), 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. It is recommended to wash the cells at least twice with a medium or buffer of choice (e.g. DMEM, IMDM, RPMI, or PBS containing 10% fetal bovine serum [FBS]) prior to labeling and separation. Filter aggregated suspensions through a 37 µm cell strainer (Catalog #27250) for optimal results.

After preparation, resuspend cells at 5 x 10^7 cells/mL in recommended medium.

LYSED LEUKAPHERESIS

1. Add 4 parts Ammonium Chloride Solution (Catalog #07800) to 1 part leukapheresis sample (e.g. Human Peripheral Blood Leukopak, Fresh*, Catalog #70500).

   NOTE: If working with large volumes (> 20 mL), concentrate leukapheresis sample first by centrifuging at 300 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original leukapheresis volume with recommended medium (e.g. for 30 mL of cells, resuspend in 3 mL of recommended medium and add 12 mL of Ammonium Chloride Solution). For small volumes (≤ 20 mL), add Ammonium Chloride Solution directly to the leukopak.
2. Incubate on ice for 15 minutes.
3. Wash the cells by topping up the tube with recommended medium. Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. OPTIONAL (FOR PLATELET REMOVAL):

   a. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 120 x g for 10 minutes at room temperature with the brake off. Carefully remove the supernatant.

   b. Repeat step 4a one or more times until most of the platelets have been removed (indicated by a clear supernatant).
5. Resuspend the cells at 5 x 10^7 cells/mL in recommended medium.

WASHED LEUKAPHERESIS

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium or PBS containing 2% FBS.

Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). If platelet removal is necessary, centrifuge at 120 x g for 10 minutes with the brake off.

Remove the supernatant and resuspend the cells at 5 x 10^7 cells/mL in recommended medium.

* Some primary cell products are available only in select regions. Contact us at techsupport@stemcell.com for further information.

** SepMate™ IVD is available only 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 pages 1 and 2 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. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	**Add Isolation Cocktail to sample.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample	50 µL/mL of sample
	Add Platelet Removal Cocktail to sample. **NOTE: Do not vortex cocktail.** **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	• 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

		EASYSEP™ MAGNETS			
STEP	INSTRUCTIONS	EasyPlate™ (Catalog#18102)	EasyEights™ (Catalog #18103) 5 mL tube	EasyEights™ (Catalog #18103) 14 mL tube	Easy50 (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. Catalog #38018)	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)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add Isolation Cocktail to sample. NOTE: Do not vortex cocktail.	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. NOTE: Do not vortex cocktail.	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	• 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 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; place the new tube (without lid) into the magnet and incubate for a second 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 EasyEights™ 5 mL tube, use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube, use a 10 mL serological pipette [Catalog #38004]).

Directions for Use – Fully Automated RoboSep™ Protocol

See pages 1 and 2 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 #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 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

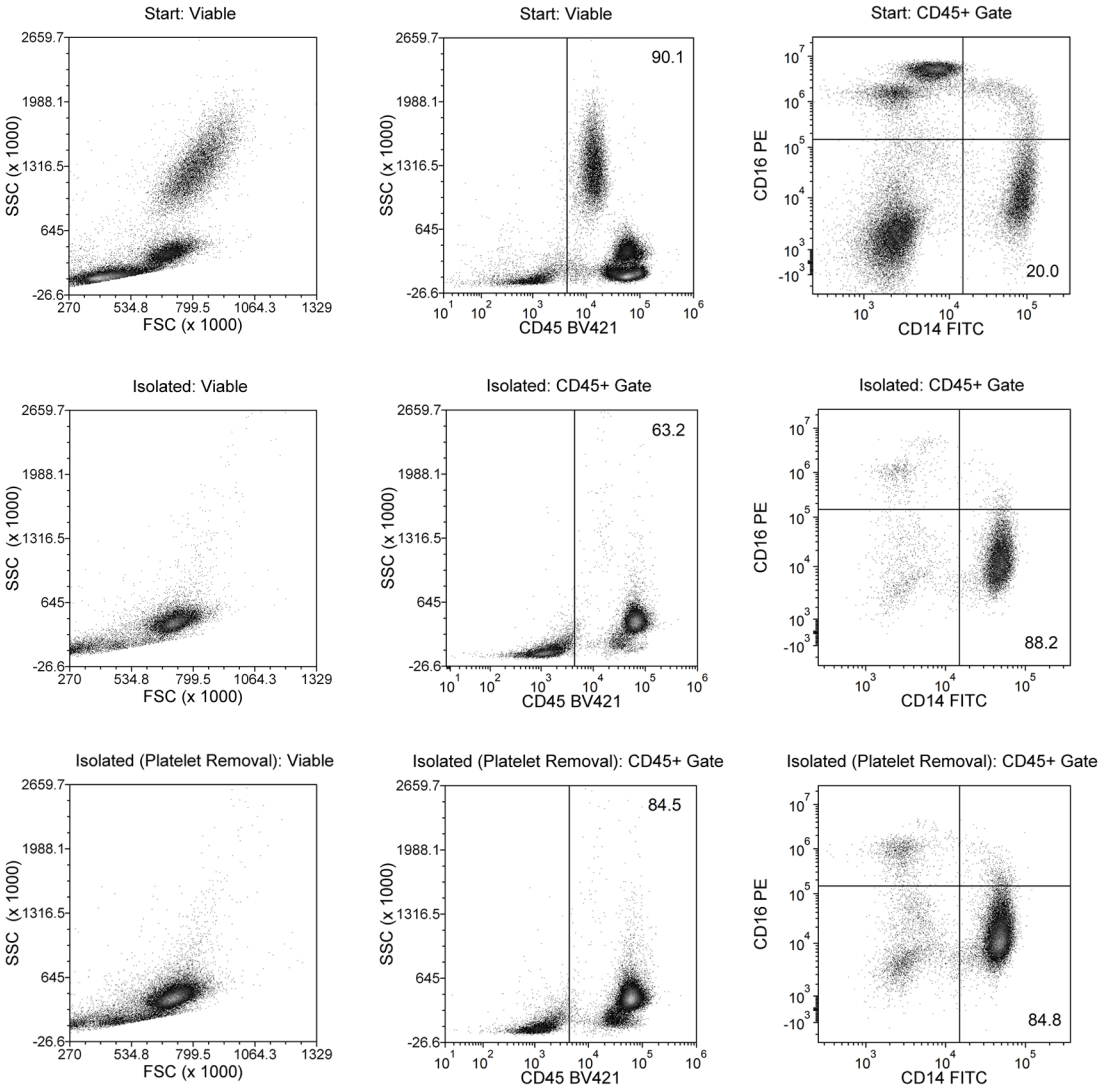
EasySep™ Human Platelet Removal Cocktail performs optimally when used with PBMCs prepared from human whole peripheral blood and following the Sample Preparation procedure for generation of PBMCs. The use of EasySep™ Human Platelet Removal Cocktail with leukapheresis samples or previously frozen PBMCs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

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),
- Anti-Human CD16 Antibody, Clone 3G8 (Catelog #60041), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018)

Data



Starting with PBMCs prepared from human whole peripheral blood, the monocyte cell content (CD14+CD16-) of the isolated fraction obtained without (middle plots) or with (bottom plots) EasySep™ Human Platelet Removal Cocktail is typically 89.7 ± 3.4% and 87.3 ± 4.5%, respectively (gated on CD45, mean ± SD using the purple EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions obtained without or with EasySep™ Human Platelet Removal Cocktail are 20%, 88.2%, 84.8%, respectively (gated on CD45) and 18%, 55.7%, and 71.7% (not gated on CD45).