EasySep™ Direct Human Monocyte Isolation Kit

Negative Selection

Catalog #19669

For processing 100 mL whole blood

STEMCELL™ TECHNOLOGIES

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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

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Description

Isolate highly purified CD14+ monocytes directly from human whole blood by immunomagnetic negative selection.

The benefits of this kit include:

- > 99.9% RBC depletion without the need for density gradient centrifugation, sedimentation, or lysis
- Up to 92% purity of isolated cells
- Fast, easy-to-use and column-free
- Isolated cells are untouched

This kit targets non-monocytes and CD16+ monocytes for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and EasySep™ Direct RapidSpheres™, and separated using an EasySep™ magnet. Desired cells are simply collected into a new tube and 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™ Direct Human Monocyte Isolation Cocktail	19669C	2 x 2.5 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™ Direct RapidSpheres™ 50300	50300	4 x 2.5 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles and monoclonal antibodies in PBS.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) and should be refrigerated upon receipt.

Precipitate may be observed in the cocktail vial but will not affect performance.

Components have been sterility tested.

Sample Preparation

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.

For best recovery, use unprocessed human whole blood. Recovery of the desired isolated cells decreases with samples that are older than 24 hours.

The volume of blood that can be processed depends on the EasySep™ magnet used for the isolation procedure. Blood samples must be placed in the required tube to properly fit into the appropriate EasySep™ magnet (see Tables 1 and 2).

BUFFY COAT (OPTIONAL - FOR USE WITH ROBOSEP™)

1. Add an equal volume of recommended medium to whole blood.
2. Centrifuge at 800 x g for 10 minutes at room temperature (15 - 25°C) with the brake off.
3. Remove the concentrated leukocyte band (this is the buffy coat), plus a small portion of the plasma and concentrated red blood cells (RBCs). The target is to concentrate the leukocytes approximately 5-fold while maintaining the same hematocrit (e.g. collect 2 mL of buffy coat when starting with 10 mL of whole blood).
4. Transfer buffy coat to the required tube (see Table 3).

Recommended Medium

PBS containing 1 mM EDTA. Medium should be free of Ca++ and Mg++. EasySep™ Buffer (Catalog #20144) may also be used.

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™ Direct Human Monocyte Isolation Kit Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 EasySep™ (Catalog #18000)	"The Big Easy" (Catalog #18001) 
1	Add whole blood sample to required tube.	0.5 - 1 mL	1 - 3 mL
	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	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds
3	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
4	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample
5	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
6	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 4X the original sample volume	Top up to 4X the original sample volume
7	Place the tube (without lid) into the magnet and incubate.	RT for 3 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	Add RapidSpheres™ to the new tube containing the enriched cells.	Use same volume as in step 4	Use same volume as in step 4
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes
10	Remove the tube from the magnet and place the tube from step 9 (without lid) into the magnet and incubate for a second separation.	RT for 3 minutes	RT for 5 minutes
11	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
12	Remove the tube from the magnet and place the new tube from step 11 (without lid) into the magnet and incubate for a third separation.	RT for 3 minutes	RT for 5 minutes
13	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)

‡ When using the maximum top-up volume the sample may extend above the top of the magnet. This will not affect performance.

* Following the first magnetic separation the collected cells may contain a significant amount of RBCs and may look similar to the original unprocessed human whole blood sample.

** To minimize RBC contamination in the isolated cells, pour off the sample along a clean area of the tube (i.e. the opposite side to where the sample was poured in).

Table 2. EasySep™ Direct Human Monocyte Isolation Kit Protocol

		EASYSEP™ MAGNETS		
		EasyEights™ (Catalog #18103)		
STEP	INSTRUCTIONS	5 mL tube	14 mL tube	Easy 50 (Catalog #18002)
1	Add whole blood sample to required tube.	0.5 - 1.5 mL	1 - 4 mL	4 - 20 mL
	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)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds	30 seconds	30 seconds
3	Add Isolation Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
4	Add RapidSpheres™ to sample.	50 µL/mL of sample	50 µL/mL of sample	50 µL/mL of sample
5	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
6	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 3X the original sample volume	Top up to 3X the original sample volume	• Top up to 3X the volume for samples ≤ 16 mL • Top up to 50 mL for samples > 16 mL
7	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 10 minutes
8	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect the entire clear fraction from top to bottom. For optimal recovery, also collect a small volume of RBCs (up to 10% of the starting sample volume).	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
9	Add RapidSpheres™ to the new tube containing the enriched cells.	Use same volume as in step 4	Use same volume as in step 4	Use same volume as in step 4
	Mix and incubate.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
10	Remove the tube from the magnet and place the tube from step 9 (without lid) into the magnet and incubate for a second separation.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
11	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction.	Use a new 5 mL tube	Use a new 14 mL tube	Use a new 50 mL tube
12	Remove the tube from the magnet and place the new tube from step 11 (without lid) containing the enriched cells into the magnet and incubate for a third separation.	RT for 5 minutes	RT for 5 minutes	RT for 5 minutes
13	Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction.	Isolated cells are ready for use	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)

*** Collect the entire enriched cell suspension, 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 page 1 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

NOTE: For RoboSep™-S, ensure the software is at least v.1.2.0.2 and RoboSep™ Direct-compatible carousel is installed. Contact us at techsupport@stemcell.com for more information.

Table 3. RoboSep™ Direct Human Monocyte Isolation Kit Protocol

STEP	INSTRUCTIONS	RoboSep™ (Catalog #21000) 
1	Prepare sample within the volume range.	**For blood:** 1 - 6 mL **For buffy coat:** 2 - 5 mL
	Add sample to required tube.	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Select protocol.	• EasySep Direct Human Monocyte Isolation 19669 - whole blood • EasySep Direct Human Monocyte Isolation 19669 - buffy coat
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Load the carousel.	Follow on-screen prompts
	Start the protocol.	Press the green “Run” button
5	Unload the carousel when the run is complete.	Isolated cells are ready for use

Notes and Tips

REMOVAL OF RESIDUAL RBCs IN THE ISOLATED CELLS

Typically, further RBC depletion is not required following cell isolation. If residual RBCs are visible in the isolated cell pellet following centrifugation after the end of the protocol, resuspend in a small volume (0.2 - 2.5 mL) of recommended medium or desired culture medium and place in a smaller EasySep™ magnet for an additional 5-minute separation. Collect the supernatant; the isolated cells are ready for use in downstream applications. Residual RBCs may also be lysed using Ammonium Chloride Solution (Catalog #07800).

ASSESSING PURITY

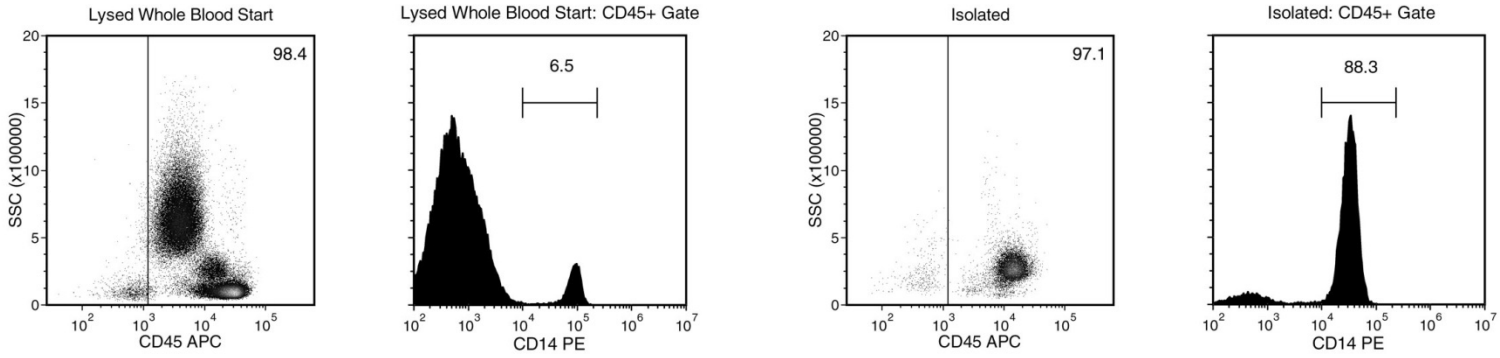
For purity assessment of monocytes (CD14+CD45+) by flow cytometry use the following fluorochrome-conjugated antibody clones:

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

  NOTE: It is recommended to assess purity on CD45-positive cells to exclude debris, platelets, and RBCs. Include a viability dye if necessary (e.g. Propidium Iodide [Catalog #75002]; 7-AAD [7- Aminoactinomycin D; Catalog #75001]).

Data

Starting with human whole blood from normal healthy donors, the typical monocyte (CD14+) content of the non-lysed final isolated fraction is 82.2 ± 8.4% (gated on CD45) or 79.0 ± 10.1% (not gated on CD45).



In the above example, the monocyte (CD14+) content of the lysed whole blood start sample and the non-lysed final isolated fraction is 6.5% and 88.3% (gated on CD45), respectively, or 6.4% and 85.7% (not gated on CD45), respectively. The starting frequency of monocytes in the non-lysed whole blood start sample above is approximately 0.007% (data not shown).