EasySep™

Negative Selection

Catalog #19058

EasySep™ Human Monocyte Enrichment Kit without CD16 Depletion

For processing 1 x 10^9 cells

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

Document #28748 | Version 4_6_1

Description

Isolate untouched and highly purified CD14+CD16+ monocytes from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or lysed leukapheresis samples by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 81% purity
- Untouched, viable cells

This kit targets non-monocytes 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.

If removal of all CD16+ cells is desired, use the EasySep™ Human Monocyte Enrichment Kit (Catalog #19059), which contains anti-CD16.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human Monocyte Enrichment Cocktail w/o CD16 Depletion	19058C.2	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

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 the need for careful sample layering, use the SepMate™-15 (Catalog #15415) or SepMate™-50 (Catalog #15450) cell isolation tube. Following density centrifugation, platelets should be removed by resuspending the cells in recommended medium and centrifuging at 120 x g for 10 minutes with the brake off. Carefully remove and discard the supernatant and repeat the wash.

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 10^7 cells/mL in recommended medium.

LEUKAPHERESIS (LEUKO PAK)

If working with large volumes (> 150 mL), concentrate leukapheresis cells first by centrifuging at 500 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 300 mL of cells, resuspend in 30 mL of recommended medium). For small volumes (≤ 150 mL), add Ammonium Chloride Solution (Catalog #07800) directly to the leukapheresis sample.

1. Add an equal volume of Ammonium Chloride Solution to the leukapheresis sample.
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) with 1 mM EDTA. Medium should be free of Ca++ and Mg++. For optimal performance, store recommended medium at 2 - 8°C prior to use.

Directions for Use – Manual EasySep™ Protocols

See page 1 for Sample Preparation and Recommended Medium. Refer to Tables 1, 2, and 3 for detailed instructions regarding the EasySep™ procedure for each magnet.

Table 1. EasySep™ Human Monocyte Enrichment Kit without CD16 Depletion 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 0.25 - 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 Enrichment Cocktail to sample.**	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 10 minutes	2 - 8°C for 10 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.	2 - 8°C for 5 minutes	2 - 8°C 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.**	Use a new 5 mL tube Isolated cells are ready for use	Use a new 14 mL tube Isolated cells are ready for use
OPTIONAL ADDITIONAL SEPARATIONS to improve purity or recovery are listed on the following page.			

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.

Table 2. EasySep™ Human Monocyte Enrichment Kit without CD16 Depletion Protocol

		EASYSEP™ MAGNETS	
STEP	**INSTRUCTIONS**	 **EasySep™ (Catalog #18000)**	**"The Big Easy" (Catalog #18001)** 
OPTIONAL ADDITIONAL SEPARATION for PURITY NOTE: This will improve purity but may reduce recovery		---	---
7	Remove the tube from the magnet and 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	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the enriched cell suspension.	Isolated cells are ready for use	Isolated cells are ready for use
OPTIONAL ADDITIONAL SEPARATION for RECOVERY NOTE: This will improve recovery but may reduce purity		---	---
7	Remove the tube from the magnet and add recommended medium to indicated volume. Mix by gently pipetting up and down 5 - 6 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
8	Place the tube (without lid) into the magnet and incubate.	RT for 2.5 minutes	RT for 2.5 minutes
9	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the enriched cell suspension.	Use a new 14 mL tube Combine with poured-off fraction from step 6 Isolated cells are ready for use	• Use a new 14 mL tube for start samples < 2 mL • Use a new 50 mL tube for start samples ≥ 2 mL Combine with poured-off fraction from step 6 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.

Table 3. EasySep™ Human Monocyte Enrichment Kit without CD16 Depletion Protocol

		EASYSEP™ MAGNETS	
STEP	INSTRUCTIONS	 **EasyPlate™ (Catalog #18102)**	**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 1 - 40 mL
	Add sample to required tube (or plate when using the EasyPlate™ EasySep™ Magnet).	Round bottom, non-tissue culture-treated 96-well plate (e.g. Costar Catalog #3788)	50 mL conical tube (e.g. Corning Catalog #352070)
3	Add Enrichment Cocktail to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 10 minutes	2 - 8°C for 10 minutes
4	Vortex Magnetic Particles.	30 seconds	30 seconds
5	Add Magnetic Particles to sample.	50 µL/mL of sample	50 µL/mL of sample
	Mix and incubate.	2 - 8°C for 5 minutes	2 - 8°C for 10 minutes
6	Add recommended medium to top up sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	Top up to 0.25 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 10 minutes
8	Carefully pipette** (do not pour) the enriched cell suspension into a new tube or plate.	Isolated cells are ready for use	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
** Collect the entire supernatant, all at once, into a single pipette.

Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 4 for detailed instructions regarding the RoboSep™ procedure.

Table 4. RoboSep™ Human Monocyte Enrichment Kit without CD16 Depletion 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	**Select protocol.**	Human Monocyte Negative Selection 19058-high recovery
3	**Vortex Magnetic Particles.**	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

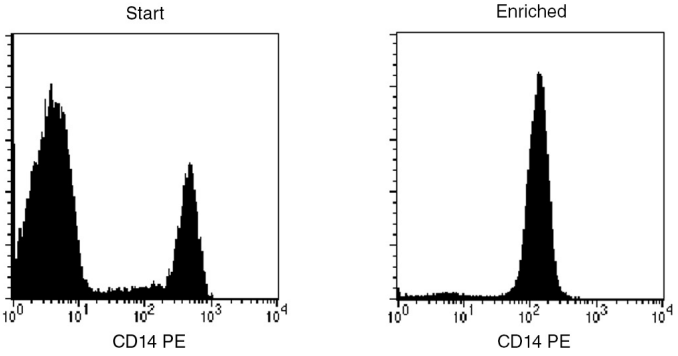
Notes and Tips

ASSESSING PURITY

For purity assessment of monocytes by flow cytometry use the following fluorochrome-conjugated antibodies:

- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004)
- Anti-Human CD16 Antibody, Clone 3G8 (Catalog #60041; optional)

Data



Starting with freshly prepared PBMCs, the CD14+ cell content of the enriched fraction typically ranges from 73 - 81%. Slightly lower CD14+ cell purities may be obtained from samples that contain a large number of CD16+ cells. In the example above, the final purities of the start and enriched fractions are 14% and 80%, respectively.