

EasySep™ Human Monocyte Enrichment Kit without CD16 Depletion

For processing 1 x 10⁹ cells

Catalog #19058
#19058RF RoboSep™

Negative Selection

Document # 1000000855 | Version 03



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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 lysed leukapheresis samples by immunomagnetic negative selection.

- Fast, easy-to-use, and column-free
- Up to 89% 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 the depletion of CD16+ cells is desired, use EasySep™ Human Monocyte Enrichment Kit (Catalog #19059) or EasySep™ Human Monocyte Isolation Kit (Catalog #19359), which contain 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. Includes an Fc receptor blocking antibody.
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 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 37 µm cell strainer (Catalog #27250) 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).

LYSED LEUKAPHERESIS

1. Add 4 parts Ammonium Chloride Solution (Catalog #07800) to 1 part leukapheresis sample.
NOTE: If working with large volumes (> 20 mL), concentrate the Leukopak first by centrifuging at 300 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original Leukopak 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 the cells 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 10⁷ 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 - 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. Catalog #38007)	14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)
2	Add Enrichment Cocktail to sample. NOTE: Do not vortex cocktail.	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. 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.	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	<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.	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; 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 the enriched cell suspension into a new tube.	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	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • 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. Catalog #38018)	50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010)
2	Add Enrichment Cocktail to sample. NOTE: Do not vortex cocktail.	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. 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.	2 - 8°C for 5 minutes	2 - 8°C for 10 minutes
5	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	<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 10 minutes
6	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 #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	Select protocol.	Human Monocyte Negative Selection 19058-high recovery	
3	Vortex Magnetic Particles. 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	

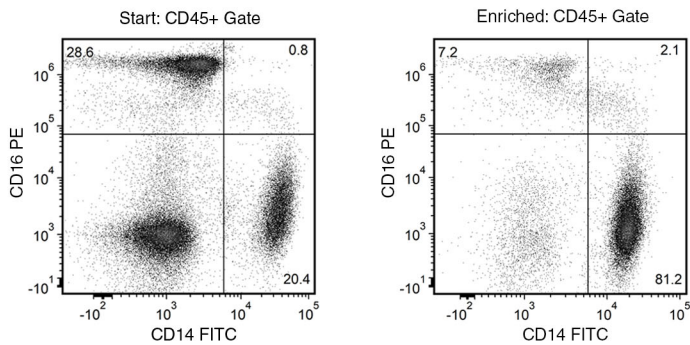
Notes and Tips

ASSESSING PURITY

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

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

Data



Starting with freshly prepared PBMCs, the CD14+ cell content of the enriched fraction is 82.7 ± 6.7% (mean ± SD using the purple EasySep™ magnet). Slightly lower CD14+ cell purities may be obtained from samples that contain a large number of CD16+ cells. In the above example, the start and final enriched fractions are 21.2% and 83.3%, respectively.

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