EasySep™ Human Monocyte Enrichment Kit without CD16 Depletion

For processing 1 x 10^10 cells using the Easy 250 EasySep™ Magnet

Catalog #100-1525

Negative Selection

Document #10000025884 | Version 01

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+/- and CD14-CD16+CD56- monocytes from fresh lysed leukapheresis samples by immunomagnetic negative selection.

- Fast, easy-to-use, and column-free
- Up to 90% 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 the EasySep™ Human Monocyte Isolation Kit (Catalog #100-0697), which contains anti-CD16.

NOTE: This is the Product Information Sheet (PIS) for isolating CD14+CD16+/- and CD14-CD16+CD56- monocytes using the Easy 250 EasySep™ Magnet (Catalog #100-0821). If using other magnets, refer to the applicable PIS, available at www.stemcell.com or contact us to request a copy.

Component Descriptions

COMPONENT NAME	COMPONENT #	QUANTITY	STORAGE	SHELF LIFE	FORMAT
EasySep™ Human Monocyte Enrichment Cocktail w/o CD16 Depletion	300-0970	1 x 10 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	300-0393	1 x 10 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles in water.

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) but should be stored as indicated above.

Sample Preparation

For available fresh samples, see www.stemcell.com/primarycells.

NOTE: Working with fresh lysed leukapheresis samples is recommended for optimal performance.

LYSED LEUKAPHERESIS

1. Add an equal volume of Ammonium Chloride Solution (Catalog #07800) to the Leukopak (e.g. Human Peripheral Blood Leukopak, Fresh, Catalog #70500*). NOTE: If working with large volumes (> 150 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 300 mL of cells, resuspend in 30 mL of recommended medium and add 30 mL of Ammonium Chloride Solution). For small volumes (≤ 150 mL), add Ammonium Chloride Solution directly to the Leukopak.
2. Incubate on ice for 15 minutes.
3. Centrifuge at 300 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 120 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.

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

Recommended Medium

EasySep™ Buffer (Catalog #20144), or PBS containing 2% fetal bovine serum 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 Table 1 for detailed instructions regarding the EasySep™ procedure.

Table 1. EasySep™ Human Monocyte Enrichment Kit without CD16 Depletion Protocol

		EASYSEP™ MAGNETS
STEP	INSTRUCTIONS	**Easy 250 (Catalog #100-0821)** 
1	**Prepare sample at the indicated cell concentration within the volume range.**	5 x 10^7 cells/mL 40 - 225 mL
	Add sample to required flask.	T-75 cm^2 cell culture flask (i.e. Catalog #200-0500)
2	**Add Enrichment Cocktail to sample.** **NOTE: Do not vortex cocktail.**	50 µL/mL of sample
	Mix and incubate (see Notes and Tips).	RT for 10 minutes†
3	**Vortex Magnetic Particles.** **NOTE: Particles should appear evenly dispersed.**	30 seconds
4	**Add Magnetic Particles to sample.**	50 µL/mL of sample
	Mix and incubate.	RT 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 100 mL for samples < 80 mL • Top up to 250 mL for samples ≥ 80 mL
	Place the flask (without cap) into the magnet and incubate.	RT for 10 minutes
6	**Carefully pipette (do not pour) the supernatant into a new tube or centrifuge bottles.**	Use a new tube or centrifuge bottle*
7	**Centrifuge sample; carefully aspirate and discard supernatant.**	Centrifuge at 300 x g for 10 minutes at RT with low brake
	Resuspend to the desired cell concentration using recommended medium.	Isolated cells are ready for use

RT - room temperature (15 - 25°C)
† For higher recovery, incubate at 2 - 8°C. For more information, see Notes and Tips.
* e.g. 50 mL (30 x 115 mm) conical tube (Catalog #38010) or 225 mL centrifuge bottle (Corning Catalog #352075)

Notes and Tips

- After the addition of the cocktails and magnetic particles, mix the sample with a 25 mL or 50 mL serological pipette (e.g. Catalog #38005/38006).
  NOTE: Mixing can also be performed by rotating or gently agitating the flask. Cap the flask first to prevent spilling.
- To collect the supernatant, gently sweep the pipette back and forth along the midline of the T-75 cm^2 flask while aspirating. Avoid touching the sides of the flask. Switch to a 10 mL or smaller serological pipette to collect the residual supernatant. Removal of the residual supernatant is required in order to obtain high purity.
- The recommended temperature for the isolation is dependent on whether higher purity or recovery is preferred:
  - For higher purity, perform the isolation at room temperature (15 - 25°C).
  - For higher recovery, perform the isolation at 2 - 8°C.

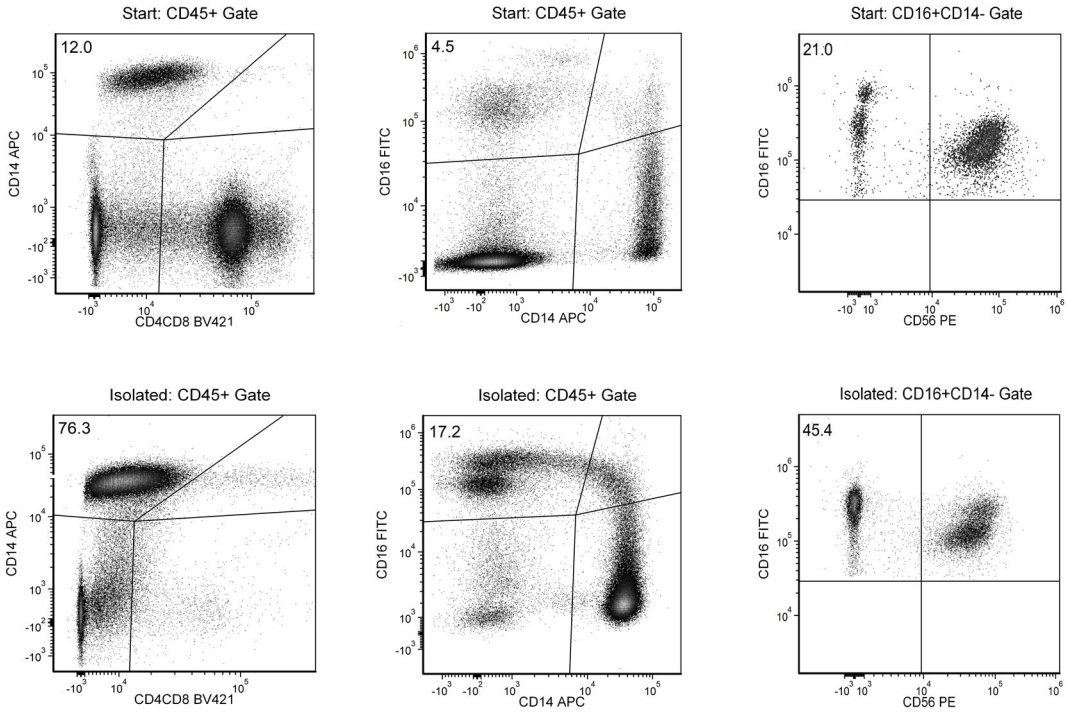
ASSESSING PURITY

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

- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004), and
- Anti-Human CD16 Antibody, Clone 3G8 (Catalog #60041), and
- Anti-Human CD56 Antibody, Clone HCD56 (Catalog #60021), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018, and
- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog#60011), or
- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016), and
- Anti-Human CD8a Antibody, Clone RPA-T8 (Catalog #60022)

NOTE: Include a viability dye (e.g. Propidium Iodide [Catalog #75002] or 7-AAD [7- Aminoactinomycin D; Catalog #75001]).

Data



Starting with lysed leukopheresis samples, the monocyte cell content ([CD14+CD16+/-] plus [CD16+CD14-] x [CD16+CD14-CD56-]) of the isolated fraction is typically 86.6 ± 3.6% (gated on CD45+ cells, mean ± SD for the Easy 250 EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions obtained are 12.9% and 84.1%, respectively.