EasySep™ Human CD14 Positive Selection Kit II

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

Catalog #100-0694

Positive Selection

Document #10000011822 | Version 02

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 highly purified CD14+ cells from fresh leukapheresis samples by immunomagnetic positive selection.

- Fast and easy-to-use
- Up to 99% purity with high recovery
- No columns required

This kit targets CD14+ cells for positive selection with antibodies recognizing the CD14 surface marker. Desired cells are labeled with antibodies and magnetic particles and separated without columns using an EasySep™ magnet. Unwanted cells are simply pipetted off, while desired cells remain in the flask. Isolated cells are immediately available for downstream applications, such as flow cytometry, culture, or DNA/RNA extraction.

NOTE: This is the Product Information Sheet (PIS) for isolating CD14+ cells 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 CD14 Positive Selection Cocktail II	300-0297	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 with 10% HPCD and 0.09% rHA.
EasySep™ Dextran RapidSpheres™ 50103	50103	2 x 1 mL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	A suspension of magnetic particles in water.

HPCD - 2-hydroxypropyl-β-cyclodextrin; PBS - phosphate-buffered saline; rHA - recombinant human albumin

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. Alternatively, washed leukapheresis samples may be used (see below) for faster sample processing, but a reduction in performance may be observed.

LYSED LEUKAPHERESIS

1. Concentrate the Leukopak (e.g. Human Peripheral Blood Leukopak, Fresh, Catalog #70500*) 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 the recommended medium (e.g. for 200 mL of cells, resuspend in 20 mL of the recommended medium).
2. Add 4 parts Ammonium Chloride (Catalog #07800) to 1 part leukapheresis sample (e.g. for 20 mL of concentrated cells, add 80 mL of Ammonium Chloride Solution).
3. Incubate on ice for 15 minutes.
4. Wash the cells by topping up the tube with the recommended medium. Centrifuge at 300 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
5. OPTIONAL (FOR PLATELET REMOVAL):
   a. Wash the cells by topping up the tube with the 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 5a one or more times until most of the platelets have been removed (indicated by a clear supernatant).
6. Resuspend the cells at 1 x 10^8 cells/mL in the recommended medium.

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

WASHED LEUKAPHERESIS

Wash the fresh peripheral blood leukapheresis sample (e.g. Human Peripheral Blood Leukopak, Fresh) by adding an equivalent volume of the recommended medium or PBS containing 2% fetal bovine serum (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 1 x 10^8 cells/mL in the recommended medium.

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 Table 1 for detailed instructions regarding the EasySep™ procedure.

Table 1. EasySep™ Human CD14 Positive Selection Kit II Protocol

STEP	INSTRUCTIONS	Easy 250 EasySep™ Magnet (Catalog #100-0821) 
1	Prepare sample at the indicated cell concentration within the volume range.	1 x 10^8 cells/mL 40 - 125 mL
	Add sample to required flask.	T-75 cm^2 cell culture flask (i.e. Corning Catalog #353135)
2	Add Selection Cocktail to sample. NOTE: Do not vortex cocktail.	100 µL/mL of sample
	Mix and incubate (see Notes and Tips).	RT for 10 minutes
3	Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed.	30 seconds
4	Add RapidSpheres™ to sample.	12 µL/mL of sample
	Mix and incubate (see Notes and Tips).	RT for 3 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 double the original sample volume
	Place the flask (without cap) into the magnet and incubate.	RT for 10 minutes
6	Carefully pipette (do not pour) off the supernatant (see Notes and Tips). Remove the flask, containing the isolated cells, from the magnet.	Discard supernatant
7	Repeat steps as indicated. Be sure to resuspend the cells from the side of the flask.	Steps 5 and 6, two more times (total of 3 x 10-minute separations)
8	Resuspend cells in desired medium. Be sure to resuspend the cells from the side of the flask. Carefully pipette (do not pour) the cell suspension into a new tube or centrifuge bottle.	Use a new tube or centrifuge bottle*
9	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)
* e.g. 50 mL (30 x 115 mm) conical tube (Catalog #38010) or 225 mL centrifuge bottle (Corning Catalog #352075)

Notes and Tips

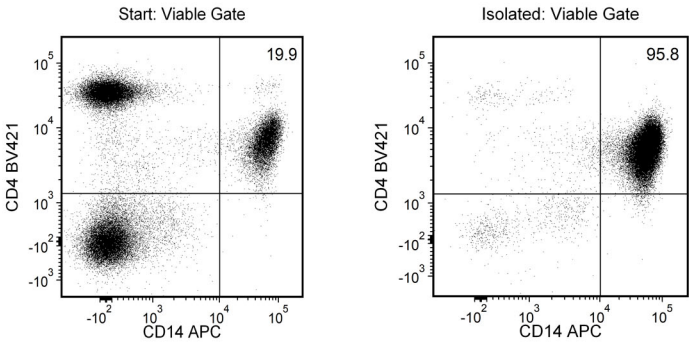
- After addition of Cocktail and RapidSpheres™, mix the sample with a 25 mL or 50 mL serological pipette (e.g. Catalog #38005/38006).
  NOTE: Mixing can also be performed by rotating or gently agitating the flask. Cap the flask first to prevent spillage.
- To remove the supernatant, gently sweep the 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.

ASSESSING PURITY

For purity assessment of CD14+ monocytes by flow cytometry, use one of the following fluorochrome-conjugated antibodies:

- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004), or
- Anti-Human CD14 Antibody, Clone MoP9 (Catalog #60124), or
- Anti-human CD14 antibody, clone UCHM1

Data



Starting with washed or lysed leukapheresis samples, the CD14+ cell content of the isolated fraction is typically 93.9 ± 4.9% (gated on viable cells, mean ± SD for the Easy 250 EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 19.9% and 95.8%, respectively.