



Positive Selection  
Catalog #18088

## EasySep™ Human Buffy Coat CD14 Positive Selection Kit

For processing 60 mL whole blood



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## Description

Isolate highly purified CD14+ cells from buffy coat samples by immunomagnetic positive selection.

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

This kit targets CD14+ cells for positive selection with an antibody 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 poured off, while desired cells remain in the tube. Isolated cells 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™ Human Buffy Coat CD14+ Positive Selection Cocktail	18088C	3 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™ Whole Blood Magnetic Nanoparticles	18180	3 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.
EasySep™ Red Blood Cell Lysis Buffer, 10X Concentrate	20110	1 x 10 mL	Store at 15 - 25°C. Do not freeze.	Stable until expiry date (EXP) on label.	A 10X concentrated red blood cell lysis reagent.

PBS - phosphate-buffered saline

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

## Additional Reagent Stability Information

REAGENT NAME	STORAGE	SHELF LIFE
EasySep™ Red Blood Cell Lysis Buffer (1X dilution)	Store at 2 - 8°C. Do not freeze.	Stable for up to 3 months. Do not exceed the expiry date (EXP) of the original component.

## Sample Preparation

For available fresh and frozen samples, see [www.stemcell.com/primarycells](http://www.stemcell.com/primarycells).

### BUFFY COAT

1. Add an equal volume of recommended medium to whole blood.

NOTE: The sample must be washed before use to remove donor-specific soluble serum factor(s) that can cause cross-linking with magnetic nanoparticles.

2. Centrifuge at 200 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.
4. Transfer a maximum of 4.5 mL of buffy coat to the required tube (see Table 1).


## Recommended Medium

EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or PBS containing 2% fetal bovine serum (FBS) 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 Buffy Coat CD14 Positive Selection Kit Protocol**

		“THE BIG EASY” EASYSEP™ MAGNET (CATALOG #18001)	
STEP	INSTRUCTIONS	Buffy Coat	
1	Prepare sample within the volume range.	Up to 4.5 mL	
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)	
2	Add 1X EasySep™ RBC Lysis Buffer to sample.	Equal volume to sample	
3	Add Selection Cocktail to sample.	50 µL/mL of diluted sample	
	Mix and incubate.	RT for 15 minutes	
4	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times	
5	Add Magnetic Particles to sample.	50 µL/mL of diluted sample	
	Mix and incubate.	RT for 10 minutes	
6	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<ul style="list-style-type: none"> <li>• Top up to 5 mL for samples &lt; 2.5 mL</li> <li>• Top up to 10 mL for samples ≥ 2.5 mL</li> </ul>	
	Place the tube (without lid) into the magnet and incubate.	RT for 10 minutes	
7	Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells.	Discard supernatant	
8	Add recommended medium to top up the sample to the indicated volume. Mix by gently pipetting up and down 2 - 3 times.	<ul style="list-style-type: none"> <li>• Top up to 5 mL for samples &lt; 2.5 mL</li> <li>• Top up to 10 mL for samples ≥ 2.5 mL</li> </ul>	
	Place the tube (without lid) into the magnet and incubate.	RT for 5 minutes	
9	Repeat steps as indicated.	Steps 7 and 8 (total of 1 x 10-minutes and 2 x 5-minute separations)	
10	Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube.	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.

## Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 2 for detailed instructions regarding the RoboSep™ procedure.

**Table 2. RoboSep™ Human Buffy Coat CD14 Positive Selection Kit Protocol**

STEP	INSTRUCTIONS	RoboSep™ (Catalog #20000 and #21000)	
1	Prepare sample within the volume range.	0.25 - 4.5 mL	
	Add sample to required tube.	14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057)	
2	Add 1X EasySep™ RBC Lysis Buffer to sample.	Equal volume to sample	
3	Select protocol.	Human CD14 Buffy Coat Positive Selection 18088-high purity	
4	Mix Magnetic Particles. NOTE: Particles should appear evenly dispersed.	Pipette up and down more than 5 times	
5	Load the carousel.	Follow on-screen prompts	
	Start the protocol.	Press the green “Run” button	
6	Unload the carousel when the run is complete. Remove the tube containing the isolated cells and resuspend in desired medium. Be sure to collect cells from the sides of the tube.	Isolated cells are ready for use	

## Notes and Tips

### EASYSEP™ RED BLOOD CELL LYSIS BUFFER

EasySep™ Red Blood Cell Lysis Buffer is supplied as a 10X concentrate. Prepare 1X lysis buffer at least 1 hour before use by adding 1 part 10X lysis buffer to 9 parts distilled or Type 1 water. Mix gently and completely before use.

### ASSESSING PURITY

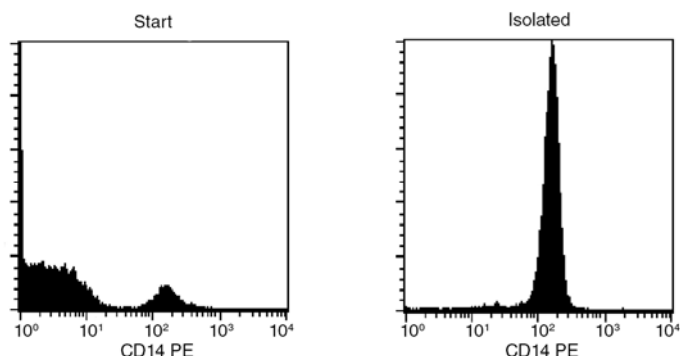
For purity assessment of CD14+ cells by flow cytometry use one of the following fluorochrome-conjugated antibody clones:

- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004)
- Anti-Human CD14 Antibody, Clone MoP9 (Catalog #60124)

One of the following methods can also be used:

- Use an alternative marker such as fluorochrome-conjugate Anti-Human CD36 Antibody, Clone FA6-152 (Catalog #60084).
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

## Data



Starting with buffy coat, the CD14+ cell content of the isolated fraction typically ranges from 96.5 - 99.6%. In the above example, the purities of the start and final isolated fractions are 11.5% and 99.6%, respectively.

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