



**EasySep™ HLA Chimerism Whole Blood CD4 Positive Selection Kit**

Positive Selection  
Catalog #17888

For processing 60 mL buffy coat or whole blood



Scientists Helping Scientists™ | [WWW.STEMCELL.COM](http://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

Document #DX22383 | Version 1\_0\_0

## Description

Isolate highly purified CD4+ cells from fresh human whole blood or buffy coat by immunomagnetic positive selection.

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

This kit targets CD4+ cells for positive selection with an antibody recognizing the CD4 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 for lineage-specific chimerism analysis.

## Component Descriptions

| COMPONENT NAME   | COMPONENT # | QUANTITY  | STORAGE                          | SHELF LIFE                               | FORMAT   |
|--|-------------|-----------|----------------------------------|--|--|
| EasySep™ HLA Chimerism Whole Blood CD4 Positive Selection Cocktail | 17888C      | 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 with 10% HPCD. |
| EasySep™ Dextran RapidSpheres™ 50100                               | 50100       | 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.              | Stable until expiry date (EXP) on label. | A 10X concentrated red blood cell lysis reagent.             |

HPCD - 2-hydroxypropyl cyclodextrin; 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).

### PERIPHERAL BLOOD

Collect whole blood in a blood collection tube containing anticoagulant.

### BUFFY COAT

1. Add an equal volume of recommended medium to whole blood.
2. Centrifuge at 800 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 (e.g. collect 2 mL of buffy coat when starting with 10 mL of whole blood).
4. Transfer a maximum of 4.5 mL of buffy coat to the required tube.

Alternatively, HetaSep™ (Catalog #07906) RBC sedimentation can be used to concentrate leukocytes. Please contact us at [techsupport@stemcell.com](mailto:techsupport@stemcell.com) for further information.


## 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 Tables 1 and 2 for detailed instructions regarding the EasySep™ procedure.


**Table 1. EasySep™ HLA Chimerism Whole Blood CD4 Positive Selection Kit Protocol**

|      |  | EASYSEP™ MAGNET  |
|------|--|--|
| STEP | INSTRUCTIONS   | “The Big Easy”<br>(Catalog #18001)                                  |
| 1    | Prepare sample within the volume range.  | 1 - 4.5 mL   |
|      | Add sample to required tube.   | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)   |
| 2    | Add 1X EasySep™ RBC Lysis Buffer to sample.  | Equal volume to sample   |
| 3    | Add Selection Cocktail to sample.  | 25 µL/mL of diluted sample   |
|      | Mix and incubate.  | RT for 3 minutes   |
| 4    | Vortex RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed.   | 30 seconds   |
| 5    | Add RapidSpheres™ to sample.   | 25 µL/mL of diluted sample   |
|      | Mix and incubate.  | RT for 3 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 diluted samples ≤ 4 mL</li> <li>• Top up to 10 mL for diluted samples &gt; 4 mL</li> </ul> |
|      | Place the tube (without lid) into the magnet and incubate.   | RT for 3 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    | Repeat steps as indicated.   | Steps 6 and 7, two more times (total of 3 x 3-minute separations)  |
| 9    | 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.

**Table 2. EasySep™ HLA Chimerism Whole Blood CD4 Positive Selection Kit Protocol**

|      |  | EASYSEP™ MAGNET  |
|------|--|--|
| STEP | INSTRUCTIONS   |  <b>EasyEights™ (Catalog #18103)</b><br><b>14 mL tube</b>             |
| 1    | Prepare sample within the volume range.  | 1 - 4.5 mL   |
|      | Add sample to required tube.   | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)   |
| 2    | Add 1X EasySep™ RBC Lysis Buffer to sample.  | Equal volume to sample   |
| 3    | Add Selection Cocktail to sample.  | 25 µL/mL of diluted sample   |
|      | Mix and incubate.  | RT for 3 minutes   |
| 4    | Vortex RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed.   | 30 seconds   |
| 5    | Add RapidSpheres™ to sample.   | 25 µL/mL of diluted sample   |
|      | Mix and incubate.  | RT for 3 minutes   |
| 6    | Add recommended medium to top up 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 diluted samples &gt; 4 mL</li> <li>• Top up to 10 mL for diluted samples ≤ 4 mL</li> </ul> |
|      | Place the tube (without lid) into the magnet and incubate.   | RT for 10 minutes  |
| 7    | Carefully pipette** (do not pour) off the supernatant. Remove the tube from the magnet; this tube contains the isolated cells. | Discard supernatant  |
| 8    | Repeat steps as indicated.   | Steps 6 and 7<br>(total of 2 x 10-minute separations)  |
| 9    | 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)

\*\* Collect the entire supernatant, all at once, into a single pipette (e.g. for the EasyEights™ 14 mL tube use a 10 mL serological pipette).

## Directions for Use – Fully Automated RoboSep™ Protocol

See page 1 for Sample Preparation and Recommended Medium. Refer to Table 3 for detailed instructions regarding the RoboSep™ procedure.

**Table 3. RoboSep™ HLA Chimerism Whole Blood CD4 Positive Selection Kit Protocol**

| STEP | INSTRUCTIONS  | RoboSep™<br>(Catalog #20000 and #21000)   |  |
|------|---|---|---|
| 1    | Prepare sample within the volume range.   | 1 - 4.5 mL  |   |
|      | Add sample to required tube.  | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008)              |   |
| 2    | Add 1X EasySep™ RBC Lysis Buffer to sample.   | Equal volume to sample  |   |
| 3    | Select protocol.<br>NOTE: Enter volume.   | HLA Chimerism CD4 WB Positive Selection 17888<br>NOTE: Enter diluted sample volume. |   |
| 4    | Vortex RapidSpheres™.<br>NOTE: Particles should appear evenly dispersed.  | 30 seconds  |   |
| 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

The EasySep™ HLA Chimerism Whole Blood CD4 Positive Selection Cocktail uses an anti-CD4 antibody clone which is known to block anti-CD4 antibody clones RFT4, SK3, SK4, RPA.T4, and 13B8.2. For purity assessment by flow cytometry use the following fluorochrome-conjugated antibody clone:

- Anti-human CD4 antibody, clone L120, and
- Anti-Human CD14 Antibody, Clone M5E2 (Catalog #60004; optional), or
- Anti-Human CD14 Antibody, Clone MoP9 (Catalog #60124; optional)

One of the following methods can also be used:

- Use alternative markers such as fluorochrome-conjugated Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011) and Anti-Human CD8a Antibody, Clone RPA-T8 (Catalog #60022) to detect CD3+CD8- cells.
- Use a fluorochrome-conjugated secondary antibody, such as Goat Anti-Mouse IgG (H+L) Antibody, Polyclonal (Catalog #60138).

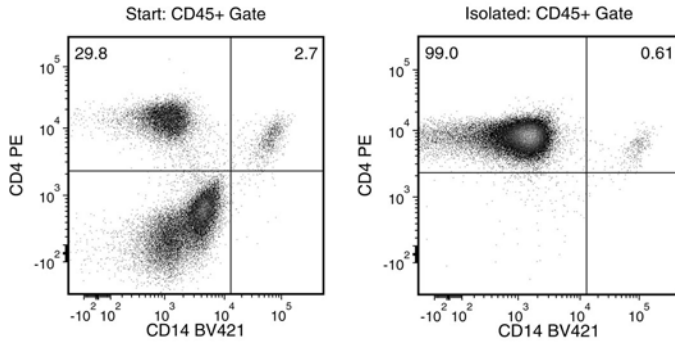
### DONOR VARIABILITY

Certain donors express one or more soluble serum factors that can cause cross-linking with magnetic particles. This may result in visible aggregates in the enriched cell fraction following positive selection. These aggregates may appear as a distinct, high side-scatter population on FSC vs. SSC plots during flow cytometry analysis of the enriched fraction. This population consists solely of particles, with no cells or platelets present, as determined by staining with fluorescently-labeled antibodies against dextran, CD41, and CD45.

Potential aggregation can be avoided by washing away the donor plasma. Dilute the sample 2-fold in the recommended medium, and centrifuge at 300 x g for 10 minutes. Remove as much plasma as possible without disturbing the white and red blood cells, then resuspend the sample to the original volume with recommended medium before beginning the separation procedure.

If the samples have not been washed, any aggregates can be gated out during flow cytometry analysis of the enriched fraction based on their FSC vs. SSC characteristics, or by their lack of CD45 expression.

Data



Starting with human whole blood, the CD4+CD14- cell content of the isolated fraction is typically  $97.0 \pm 1.8\%$  (gated on CD45; mean  $\pm$  SD using "The Big Easy" EasySep™ Magnet). In the above example, the purities of the start and final isolated fractions are 29.8% and 99.0%, respectively.

NOTE: RBCs were removed from the start sample by ammonium chloride lysis prior to flow cytometry.

STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485. PRODUCTS ARE FOR RESEARCH USE ONLY AND NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES UNLESS OTHERWISE STATED.

Copyright © 2018 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, EasyEights, EasySep, RapidSpheres, and RoboSep are trademarks of STEMCELL Technologies Canada Inc. All other trademarks are the property of their respective holders. While STEMCELL has made all reasonable efforts to ensure that the information provided by STEMCELL and its suppliers is correct, it makes no warranties or representations as to the accuracy or completeness of such information.