



EasySep™ Direct Human CD4+ T Cell Isolation Kit

Negative Selection
Catalog #19662

For processing 100 mL whole blood



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

Document #28076 | Version 1_2_1

Description

Isolate highly purified CD4+ T cells directly from human whole blood by immunomagnetic negative selection.

The benefits of this kit include:

- > 99.9% RBC depletion without the need for density gradient centrifugation, sedimentation, or lysis
- Up to 96% purity of isolated cells
- Fast, easy-to-use and column-free
- Isolated cells are untouched

This kit targets non-CD4+ T cells for removal with antibodies recognizing specific cell surface markers. Unwanted cells are labeled with antibodies and EasySep™ Direct RapidSpheres™, and separated using an EasySep™ magnet. Desired cells are simply collected into a new tube and 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™ Direct Human CD4+ T Cell Isolation Cocktail | 19662C | 2 x 2.5 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A combination of monoclonal antibodies in PBS. |
| EasySep™ Direct RapidSpheres™ 50300 | 50300 | 4 x 2.5 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A suspension of magnetic particles and monoclonal antibodies in PBS. |

PBS - phosphate-buffered saline

Components may be shipped at room temperature (15 - 25°C) and should be refrigerated upon receipt.

Precipitate may be observed in the cocktail vial but will not affect performance.

Sample Preparation

For optimal RBC depletion, collect blood using heparin or acid citrate dextrose (ACD) as an anticoagulant. The use of K2EDTA or K3EDTA as an anticoagulant is not recommended.

For best recovery, use fresh unprocessed human whole blood. Recovery of the desired isolated cells decreases with samples that are older than 24 hours.

The volume of blood samples that can be processed depends on the EasySep™ magnet used for the isolation procedure. Blood samples must be placed in the required tube to properly fit into the appropriate EasySep™ magnet (see Tables 1 and 2).



Recommended Medium

PBS (Catalog #37350) that is 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 for each magnet.

Table 1. EasySep™ Direct Human CD4+ T Cell Isolation Kit Protocol

| | | EASYSEP™ MAGNETS | |
|------|--|---|---|
| STEP | INSTRUCTIONS |  EasySep™ (Catalog #18000) |  “The Big Easy” (Catalog #18001) |
| 1 | Collect sample within the volume range. | 0.5 - 1.5 mL | 1.5 - 7 mL |
| | Add whole blood 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 | Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds | 30 seconds |
| 3 | Add Isolation Cocktail to sample. | 50 µL/mL of sample | 50 µL/mL of sample |
| 4 | Add RapidSpheres™ to sample. | 50 µL/mL of sample | 50 µL/mL of sample |
| 5 | Mix and incubate. | RT for 5 minutes | RT for 5 minutes |
| 6 | 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 double the volume for samples ≤ 5 mL • Top up to 10 mL for samples > 5 mL |
| 7 | Place the tube (without lid) into the magnet and incubate. | RT for 5 minutes | RT for 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. | Use a new 5 mL tube | Use a new 14 mL tube |
| 9 | Add RapidSpheres™ to the new tube containing the enriched cells. | Use same volume as in step 4 | Use same volume as in step 4 |
| | Mix and incubate. | RT for 5 minutes | RT for 5 minutes |
| 10 | Remove the tube from the magnet and place the tube from step 9 (without lid) into the magnet and incubate for a second separation. | RT for 5 minutes | RT for 5 minutes |
| 11 | 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 | Use a new 14 mL tube |
| 12 | Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a third separation. | --- | RT for 5 minutes |
| 13 | 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 |

RT - room temperature (15 - 25°C)

* Following the first magnetic separation, the collected cells may contain a significant amount of RBCs and may look similar to the original unprocessed human whole blood sample.

** To minimize RBC contamination in the isolated cells, pour off the sample along a clean area of the tube (i.e. the opposite side to where the sample was poured in).

Table 2. EasySep™ Direct Human CD4+ T Cell Isolation Kit Protocol

| | | EASYSEP™ MAGNETS | | |
|------|---|---|---|---|
| STEP | INSTRUCTIONS | EasyEights™ (Catalog #18103) | | Easy 50 (Catalog #18002) |
| | | 5 mL tube | 14 mL tube | |
| 1 | Collect sample within the volume range. | 0.5 - 1.5 mL | 1.5 - 7 mL | 7 - 30 mL |
| | Add whole blood 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) | 50 mL (30 x 115 mm) conical tube (e.g. Catalog #38010) |
| 2 | Vortex RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds | 30 seconds | 30 seconds |
| 3 | Add Isolation Cocktail to sample. | 50 µL/mL of sample | 50 µL/mL of sample | 50 µL/mL of sample |
| 4 | Add RapidSpheres™ to sample. | 50 µL/mL of sample | 50 µL/mL of sample | 50 µL/mL of sample |
| 5 | Mix and incubate. | RT for 5 minutes | RT for 5 minutes | RT for 5 minutes |
| 6 | 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 double the volume for samples ≤ 5 mL • Top up to 10 mL for samples > 5 mL | <ul style="list-style-type: none"> • Top up to double the volume for samples ≤ 25 mL • Top up to 50 mL for samples > 25 mL |
| 7 | Place the tube (without lid) into the magnet and incubate. | RT for 5 minutes | RT for 5 minutes | RT for 10 minutes |
| 8 | Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect the entire clear fraction from top to bottom. For optimal recovery, also collect a small volume of RBCs (up to 10% of the starting sample volume). | Use a new 5 mL tube | Use a new 14 mL tube | Use a new 50 mL tube |
| 9 | Add RapidSpheres™ to the new tube containing the enriched cells. | Use same volume as in step 4 | Use same volume as in step 4 | Use same volume as in step 4 |
| | Mix and incubate. | RT for 5 minutes | RT for 5 minutes | RT for 5 minutes |
| 10 | Remove the tube from the magnet and place the tube from step 9 (without lid) into the magnet and incubate for a second separation. | RT for 5 minutes | RT for 5 minutes | RT for 5 minutes |
| 11 | Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction. | Use a new 5 mL tube | Use a new 14 mL tube | Use a new 50 mL tube |
| 12 | Remove the tube from the magnet and place the new tube (without lid) containing the enriched cells into the magnet and incubate for a third separation. | RT for 5 minutes | RT for 5 minutes | RT for 5 minutes |
| 13 | Carefully pipette*** (do not pour) the enriched cell suspension into a new tube. NOTE: Collect only the clear fraction. | Isolated cells are ready for use | Isolated cells are ready for use | Isolated cells are ready for use |

RT - room temperature (15 - 25°C)

*** Collect the entire enriched cell suspension, all at once, into a single pipette (e.g. for EasyEights™ 5 mL tube use a 2 mL serological pipette [Catalog #38002]; for EasyEights™ 14 mL tube use a 10 mL serological pipette [Catalog #38004]).

Notes and Tips

REMOVAL OF RESIDUAL RBCs IN THE ISOLATED CELLS

Typically, further RBC depletion is not required following cell isolation. If residual RBCs are visible in the isolated cell pellet following centrifugation after the end of the protocol, resuspend in a small volume (0.2 - 2.5 mL) of recommended medium or desired culture medium and place in a smaller EasySep™ magnet for an additional 5-minute separation. Collect the supernatant; the isolated cells are ready for use in downstream applications. Residual RBCs may also be lysed using Ammonium Chloride Solution (Catalog #07800).

ASSESSING PURITY

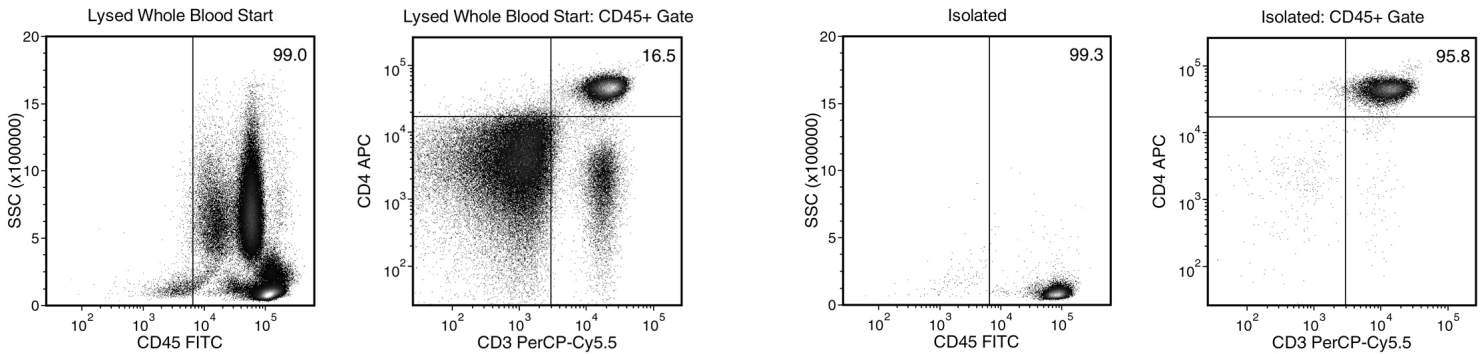
For purity assessment of CD4+ T cells (CD3+CD4+) by flow cytometry use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011), and
- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016), and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018), or Clone 2D1 (Catalog #60123)

NOTE: It is recommended to assess purity on the CD45-positive cells to exclude debris, platelets, and RBCs.

Data

Starting with human whole blood from normal healthy donors, the typical CD4+ T cell (CD3+CD4+) content of the non-lysed final isolated fraction is $93.6 \pm 2.5\%$ (gated on CD45) or $93.1 \pm 2.5\%$ (not gated on CD45).



In the above example, the CD4+ T cell (CD3+CD4+) content of the lysed whole blood start sample and non-lysed final isolated fraction is 16.5% and 95.8% (gated on CD45), respectively, or 16.3% and 95.1% (not gated on CD45), respectively. The starting frequency of CD4+ T cells in the non-lysed whole blood start sample is 0.016% (data not shown).

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 © 2017 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, EasyEights, EasySep, and RapidSpheres 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.