



Negative Selection
Catalog #19157

EasySep™ Human Memory CD4+ T Cell Enrichment Kit

For processing 1 x 10⁹ cells



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 #28945 | Version 2_3_1

Description

Isolate untouched and highly purified memory CD4+ T cells (CD4+CD45RA-CD45RO+) from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) by immunomagnetic negative selection.

- Fast, easy-to-use and column-free
- Up to 98% purity
- Untouched, viable cells

This kit targets non-memory CD4+ T cells 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.

Component Descriptions

| COMPONENT NAME | COMPONENT # | QUANTITY | STORAGE | SHELF LIFE | FORMAT |
|---|-------------|----------|-------------------------------------|--|--|
| EasySep™ Human Memory CD4+ T Cell Enrichment Cocktail | 19157C | 1 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™ D Magnetic Particles | 19250 | 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 TBS. |

PBS - phosphate-buffered saline; TBS - TRIS-buffered saline

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

Sample Preparation

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

PERIPHERAL BLOOD

Prepare a PBMC suspension from whole peripheral blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation without the need for careful sample layering, use the SepMate™-15 (Catalog #15415) or SepMate™-50 (Catalog #15450) cell isolation tube.

If using previously frozen PBMCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL for at least 15 minutes at room temperature (15 - 25°C) prior to labeling and separation. Filter clumpy suspensions through a 40 µm Cell Strainer (Catalog #27305) for optimal results.

After preparation, resuspend cells at 5 x 10⁷ cells/mL in recommended medium.



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 for each magnet.



Table 1. EasySep™ Human Memory CD4+ T Cell Enrichment Kit Protocol

| | | EASYSEP™ MAGNETS | |
|--|---|---|--|
| STEP | INSTRUCTIONS |  EasySep™ (Catalog #18000) | “The Big Easy” (Catalog #18001)  |
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 5 x 10 ⁷ cells/mL 0.25 - 2 mL | 5 x 10 ⁷ cells/mL 0.5 - 8.5 mL |
| | Add sample to required tube. | 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058) | 14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057) |
| 2 | Add Enrichment Cocktail to sample. | 50 µL/mL of sample | 50 µL/mL of sample |
| | Mix and incubate. | RT for 10 minutes | RT for 10 minutes |
| 3 | Vortex Magnetic Particles. | 30 seconds | 30 seconds |
| 4 | Add Magnetic Particles to sample. | 50 µL/mL of sample | 50 µL/mL of sample |
| | Mix and incubate. | RT for 5 minutes | RT for 5 minutes |
| 5 | 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 5 mL for samples < 2 mL • Top up to 10 mL for samples ≥ 2 mL |
| | Place the tube (without lid) into the magnet and incubate. | RT for 2.5 minutes | RT for 2.5 minutes |
| 6 | Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the enriched cell suspension into a new tube. | Use a new 14 mL tube Isolated cells are ready for use | <ul style="list-style-type: none"> • Use a new 14 mL tube for start samples < 2 mL • Use a new 50 mL tube for start samples ≥ 2 mL Isolated cells are ready for use |
| OPTIONAL ADDITIONAL SEPARATION NOTE: This will improve recovery but may reduce purity | | --- | --- |
| 7 | Remove the tube from the magnet and add recommended medium to indicated volume. Mix by gently pipetting up and down 5 - 6 times. | Top up to 2.5 mL | <ul style="list-style-type: none"> • Top up to 5 mL for samples < 2 mL • Top up to 10 mL for samples ≥ 2 mL |
| 8 | Place the tube (without lid) into the magnet and incubate. | RT for 2.5 minutes | RT for 2.5 minutes |
| 9 | Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring off the enriched cell suspension. | Combine with poured-off fraction from step 6 Isolated cells are ready for use | Combine with poured-off fraction from step 6 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™ Human Memory CD4+ T Cell Enrichment Kit Protocol

| | | EASYSEP™ MAGNETS | |
|------|---|---|---|
| STEP | INSTRUCTIONS |  EasyPlate™ (Catalog #18102) |  Easy 50 (Catalog #18002) |
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 5 x 10 ⁷ cells/mL 0.05 - 0.2 mL | 5 x 10 ⁷ cells/mL 1 - 40 mL |
| | Add sample to required tube (or plate when using the EasyPlate™ EasySep™ Magnet). | Round bottom, non-tissue culture-treated 96-well plate (e.g. Costar Catalog #3788 or Corning Catalog #351177) | 50 mL conical tube (e.g. Corning Catalog #352070) |
| 2 | Add Enrichment Cocktail to sample. | 50 µL/mL of sample | 50 µL/mL of sample |
| | Mix and incubate. | RT for 10 minutes | RT for 10 minutes |
| 3 | Vortex Magnetic Particles. | 30 seconds | 30 seconds |
| 4 | Add Magnetic Particles to sample. | 50 µL/mL of sample | 50 µL/mL of sample |
| | Mix and incubate. | RT for 5 minutes | RT for 5 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 0.25 mL | <ul style="list-style-type: none"> • Top up to 25 mL for samples ≤ 10 mL • Top up to 50 mL for samples > 10 mL |
| | Place the tube or plate (without lid) into the magnet and incubate. | RT for 10 minutes | RT for 10 minutes |
| 6 | Carefully pipette** (do not pour) the enriched cell suspension into a new tube or plate. | Isolated cells are ready for use | 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 Easy 50 EasySep™ Magnet use a 25 mL for lower volumes or 50 mL serological pipette for larger volumes).

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™ Human Memory CD4+ T Cell Enrichment Protocol

| STEP | INSTRUCTIONS | RoboSep™ (Catalog #20000 and #21000) |  |
|------|---|---|---|
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 5 x 10 ⁷ cells/mL 0.5 - 8.5 mL | |
| | Add sample to required tube. | 14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057) | |
| 2 | Select protocol. | Human Memory CD4+ T Cell Negative Selection 19157-high recovery | |
| 3 | Vortex Magnetic Particles. | 30 seconds | |
| 4 | Load the carousel. | Follow on-screen prompts | |
| | Start the protocol. | Press the green “Run” button | |
| 5 | Unload the carousel when the run is complete. | Isolated cells are ready for use | |

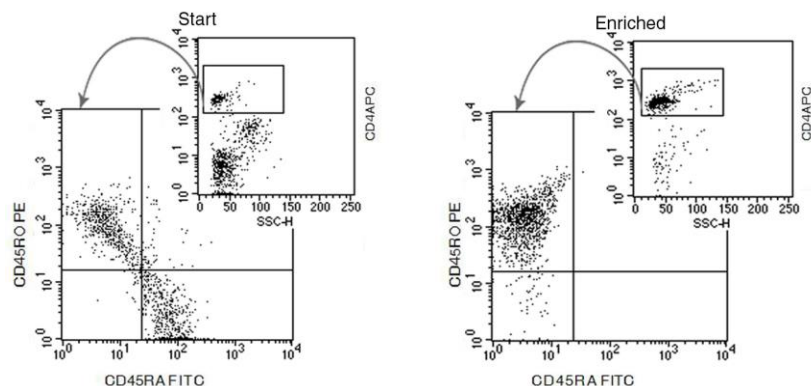
Notes and Tips

ASSESSING PURITY

For purity assessment of memory CD4+ T cells (CD4+CD45RA-CD45RO+) by flow cytometry use the following fluorochrome-conjugated antibodies:

- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016), and
- Anti-Human CD45RO Antibody, Clone UCHL1 (Catalog #60097), and
- Anti-human CD45RA antibody

Data



Starting with previously frozen mononuclear cells, the memory CD4+ T cell content (CD4+CD45RA-CD45RO+) of the enriched fraction typically ranges from 86 - 98%. In the above example, the purities of the start and final enriched fractions are 8% and 93%, respectively.

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 © 2015 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design and Scientists Helping Scientists, EasyPlate, EasySep, RoboSep, and SepMate are trademarks of STEMCELL Technologies Inc. Lymphoprep is a trademark of AXIS-SHIELD. 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.