

RoboSep™ Human CD4+CD127lowCD25+ Regulatory T Cell Isolation Kit

For processing 1 x 10⁹ cells

Catalog #18063RF

Positive Selection

Document #1000005227 | Version 00



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 human CD4+CD127lowCD25+ regulatory T cells (Tregs) from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) or leukapheresis samples.

- No-wash removal of EasySep™ Releasable RapidSpheres™
- Optional isolation of CD4+CD25- responder T cells from the same sample

First, CD25+ cells are isolated by column-free immunomagnetic positive selection using EasySep™ Releasable RapidSpheres™. Then, bound magnetic particles are removed from the EasySep™-isolated CD25+ cells and unwanted non-Tregs are targeted for depletion. The final isolated fraction contains highly purified CD4+CD127lowCD25+ Tregs that express high levels of FOXP3 and are immediately ready for downstream applications. An optional protocol allows for the isolation of CD4+CD25- responder T cells in parallel, for use in functional studies. Following cell isolation with this RoboSep™ kit, antibody complexes remain bound to the cell surface and may interact with Brilliant Violet™ antibody conjugates, polyethylene glycol-modified proteins, or other chemically related ligands.

NOTE: For manual magnet separation, refer to the Product Information Sheet for EasySep™ Human CD4+CD127lowCD25+ Regulatory T Cell Isolation Kit (Document #28053), available at www.stemcell.com or contact us to request a copy.

Component Descriptions

| COMPONENT NAME | COMPONENT # | QUANTITY | STORAGE | SHELF LIFE | FORMAT |
|---|-------------|-------------|----------------------------------|--|--|
| EasySep™ Human CD25 Positive Selection Cocktail | 18063C.1 | 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 and 0.1% BSA. Includes an Fc receptor blocking antibody. |
| EasySep™ Human CD4+ T Cell Enrichment Cocktail | 19052C.2 | 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™ Human CD127high Depletion Cocktail | 19233C | 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 and 0.1% BSA. |
| EasySep™ Releasable RapidSpheres™ 50201 | 50201 | 1 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™ Dextran RapidSpheres™ 50100 | 50100 | 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. |
| EasySep™ Release Buffer | 20145 | 2 x 1 mL | Store at 2 - 8°C. Do not freeze. | Stable until expiry date (EXP) on label. | A buffer for release of Releasable RapidSpheres™ from cells following positive selection. |
| RoboSep™ Filter Tips | 20125 | 2 x 8 Racks | Not Applicable | Not Applicable | Not Applicable |
| EasySep™ EasyTube™-14 | 20128 | 1 Bag | Not Applicable | Not Applicable | Not Applicable |

BSA - bovine serum albumin; PBS - phosphate-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 blood by centrifugation over a density gradient medium (e.g. Lymphoprep™, Catalog #07801). For more rapid PBMC preparation, use the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube.

If using previously frozen PBMCs, incubate the cells with DNase I Solution (Catalog #07900) at a concentration of 100 µg/mL at room temperature (15 - 25°C) for at least 15 minutes prior to labeling and separation. Filter aggregated suspensions through a 37 µm cell strainer (Catalog #27250) for optimal results.

After preparation, resuspend cells at 5×10^7 cells/mL in recommended medium.

* SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. In all other regions SepMate™ is available for research use only (RUO).

LEUKAPHERESIS

Wash the peripheral blood leukapheresis sample by adding an equivalent volume of recommended medium or PBS containing 2% fetal bovine serum (FBS). Centrifuge at 500 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant and resuspend the cells at 5×10^7 cells/mL in recommended medium.


Recommended Medium

EasySep™ Buffer (Catalog #20144), or PBS containing 2% FBS and 1 mM EDTA. Medium should be free of Ca++ and Mg++.

Directions for Use – Fully Automated RoboSep™ Protocols

See page 2 for Sample Preparation and Recommended Medium. Refer to Table 1 for isolating Tregs only; refer to Table 2 for isolating responder T cells and Tregs.

Table 1. RoboSep™ Human CD4+CD127lowCD25+ Regulatory T Cell Isolation Protocol

| STEP | INSTRUCTIONS | RoboSep™ (Catalog #20000 and #21000) |  |
|-------------------|--|--|---|
| PROTOCOL 1 | | | |
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 5 x 10 ⁷ cells/mL 1 - 6 mL | |
| | Add sample to required tube. | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008) | |
| 2 | Select protocol. | Human CD25+ Selection 18063 | |
| 3 | Vortex Releasable RapidSpheres™ and Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds | |
| 4 | Load the carousel. NOTE: Refer to Figure 1. | Follow on-screen prompts NOTE: When prompted to load a separation tube, place EasySep™ EasyTube™-14 into the magnet. | |
| | Start the protocol. | Press the green “Run” button | |
| 5 | Unload the carousel when the run is complete. NOTE: Dispose of biological waste from 50 mL conical tubes in positions G and H (Figure 1) before beginning Protocol 2. | Use sample in EasySep™ EasyTube™-14 in position F (Figure 1) as starting sample for Protocol 2. Continue to Protocol 2. | |
| PROTOCOL 2 | | | |
| 6 | Select protocol. | Human CD4+CD127lowCD25+ Regulatory T Cell Isolation 18063 | |
| 7 | Vortex sample in EasySep™ EasyTube™-14 from Protocol 1. | 30 seconds | |
| 8 | Vortex Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds | |
| 9 | Load the carousel. NOTE: Refer to Figure 2. | Follow on-screen prompts NOTE: Use sample in EasySep™ EasyTube™-14 from Protocol 1 as starting sample for Protocol 2. | |
| | Start the protocol. | Press the green “Run” button | |
| 10 | Unload the carousel when the run is complete. | Isolated cells (regulatory T cells) are ready for use in the 50 mL conical tube in position H (Figure 2) | |

RoboSep™ Carousel Loading Guides – Regulatory T Cells Only

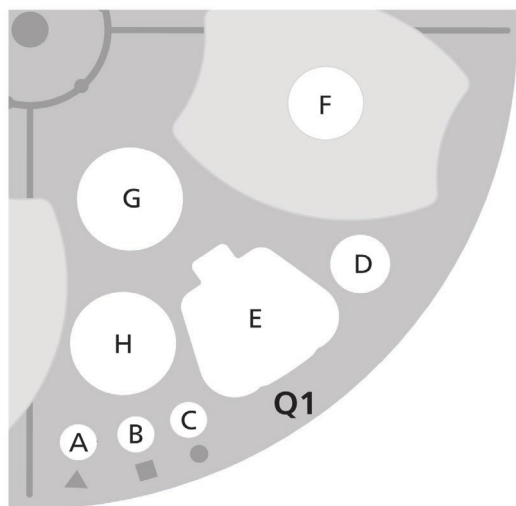


Figure 1. Regulatory T Cells Only - Protocol 1

- A - Triangle - EasySep™ Releasable RapidSpheres™ 50201
- B - Square - EasySep™ Human CD4+ T Cell Enrichment Cocktail
- C - Circle - EasySep™ Human CD25 Positive Selection Cocktail
- D - Start sample in 14 mL polystyrene round-bottom tube
- E - RoboSep™ Filter Tip rack
- F - EasySep™ EasyTube™-14 (will be start sample for Protocol 2)
- G - 50 mL conical tube (waste)
- H - 50 mL conical tube (waste)

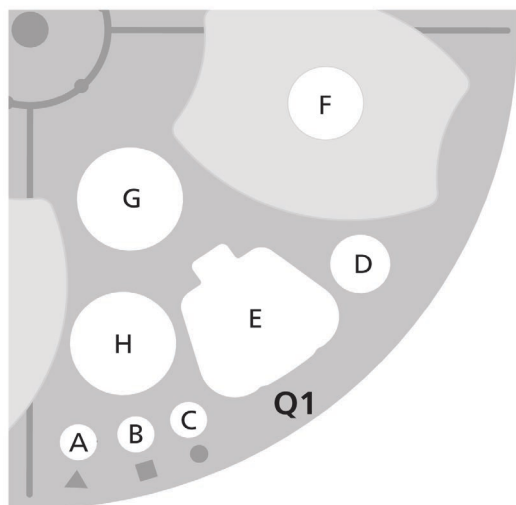



Figure 2. Regulatory T Cells only - Protocol 2

- A - Triangle - EasySep™ Dextran RapidSpheres™ 50100
- B - Square - EasySep™ Human CD127high Depletion Cocktail
- C - Circle - EasySep™ Release Buffer
- D - Start sample (EasySep™ EasyTube™-14 from Protocol 1)
- E - RoboSep™ Filter Tip rack
- F - 14 mL polystyrene round-bottom tube
- G - 50 mL conical tube (waste)
- H - 50 mL conical tube (for CD4+CD127lowCD25+ regulatory T cells)

Table 2. RoboSep™ Human CD4+CD25- Responder T Cell and CD4+CD127lowCD25+ Regulatory T Cell Isolation Protocol

| STEP | INSTRUCTIONS | RoboSep™ (Catalog #20000 and #21000) |  |
|-------------------|--|--|---|
| PROTOCOL 1 | | | |
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 5 x 10 ⁷ cells/mL 1 - 6 mL | |
| | Add sample to required tube. | 14 mL (17 x 95 mm) polystyrene round-bottom tube (e.g. Catalog #38008) | |
| 2 | Select protocol. | Human CD25+ Positive Selection with CD4+ Responder T Cell Isolation 18063 | |
| 3 | Vortex Releasable RapidSpheres™ and Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds | |
| 4 | Load the carousel. NOTE: Refer to Figure 3. | Follow on-screen prompts NOTE: When prompted to load a separation tube, place EasySep™ EasyTube™-14 into the magnet. | |
| | Start the protocol. | Press the green "Run" button | |
| 5 | Unload the carousel when the run is complete. NOTE: Dispose of biological waste from 50 mL conical tube in position G (Figure 3) before beginning Protocol 2. | Isolated responder T cells can now be collected from the 50 mL conical tube in position M (Figure 3). Use sample in EasySep™ EasyTube™-14 in position F (Figure 3) as starting sample for Protocol 2. Continue to Protocol 2. | |
| PROTOCOL 2 | | | |
| 6 | Select protocol. | Human CD4+CD127lowCD25+ Regulatory T Cell Isolation 18063 | |
| 7 | Vortex sample in EasySep™ EasyTube™-14 from Protocol 1. | 30 seconds | |
| 8 | Vortex Dextran RapidSpheres™. NOTE: Particles should appear evenly dispersed. | 30 seconds | |
| 9 | Load the carousel. NOTE: Refer to Figure 4. | Follow on-screen prompts NOTE: Use sample in EasySep™ EasyTube™-14 from Protocol 1 as starting sample for Protocol 2. | |
| | Start the protocol. | Press the green "Run" button | |
| 10 | Unload the carousel when the run is complete. | Isolated cells (regulatory T cells) are ready for use in the 50 mL conical tube in position H (Figure 4) | |

RoboSep™ Carousel Loading Guides – Regulatory T Cells and Responder T Cells

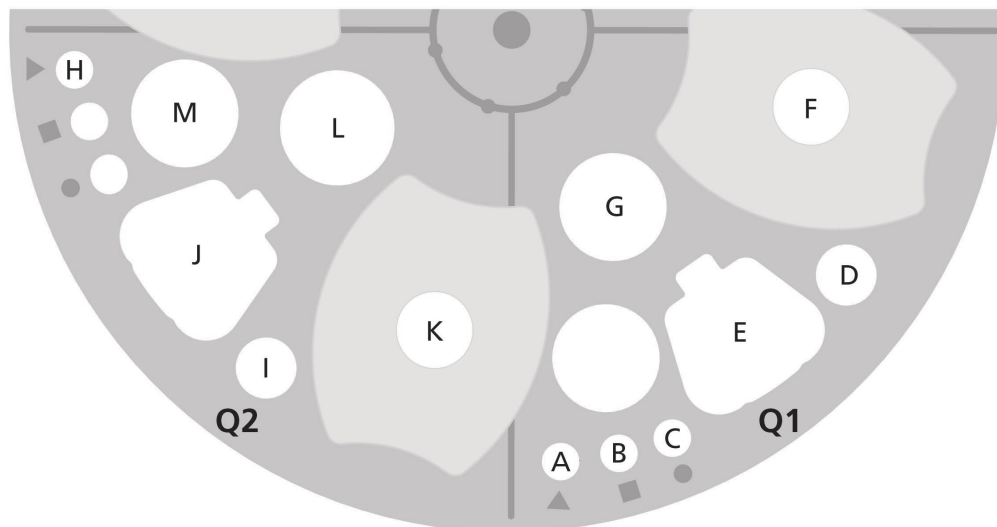


Figure 3. RoboSep™ Regulatory T Cells and Responder T Cells - Protocol 1

- A - Triangle - EasySep™ Releasable RapidSpheres™ 50201
- B - Square - EasySep™ Human CD4+ T Cell Enrichment Cocktail
- C - Circle - EasySep™ Human CD25 Positive Selection Cocktail
- D - Start sample in 14 mL polystyrene round-bottom tube
- E - RoboSep™ Filter Tip rack
- F - EasySep™ EasyTube™-14 (will be start sample for Protocol 2)
- G - 50 mL conical tube (waste)
- H - Triangle - EasySep™ Dextran RapidSpheres™ 50100
- I - 14 mL polystyrene round-bottom tube
- J - RoboSep™ Filter Tip rack
- K - 14 mL polystyrene round-bottom tube
- L - 50 mL conical tube (waste)
- M - 50 mL conical tube (for CD4+CD25- responder T cells)

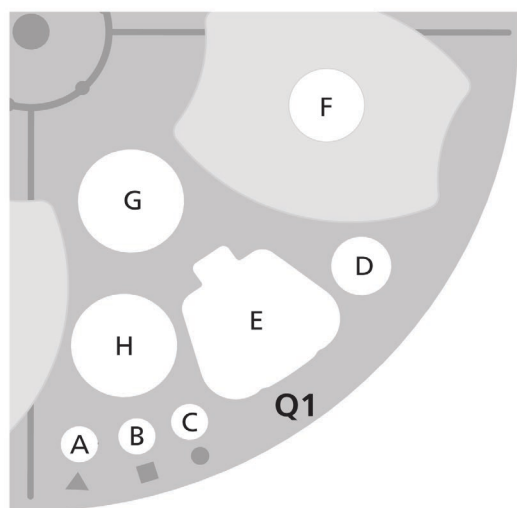


Figure 4. Regulatory T Cells and Responder T Cells - Protocol 2

- A - Triangle - EasySep™ Dextran RapidSpheres™ 50100
- B - Square - EasySep™ Human CD127high Depletion Cocktail
- C - Circle - EasySep™ Release Buffer
- D - Start sample (EasySep™ EasyTube™-14 from Protocol 1)
- E - RoboSep™ Filter Tip rack
- F - 14 mL polystyrene round-bottom tube
- G - 50 mL conical tube (waste)
- H - 50 mL conical tube (for CD4+CD127lowCD25+ regulatory T cells)

Notes and Tips

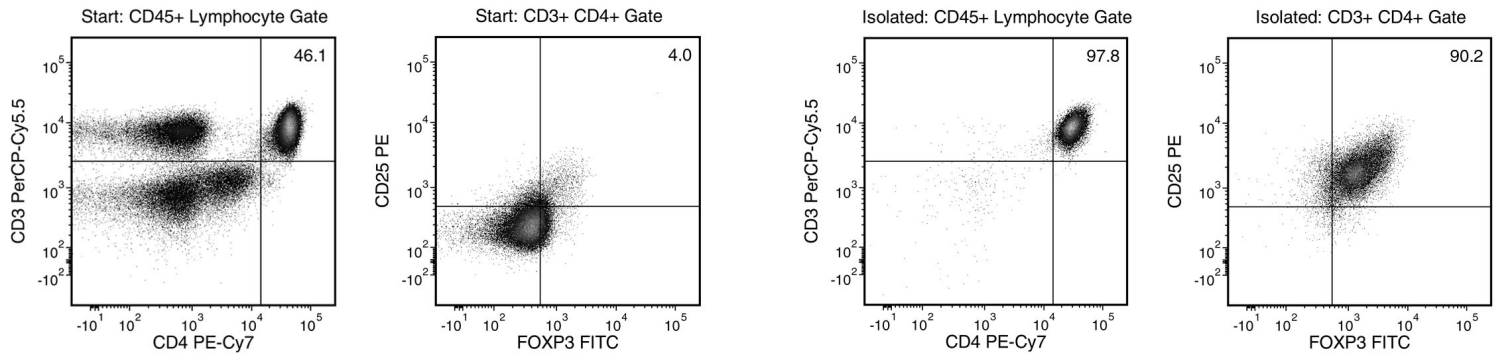
ASSESSING PURITY

EasySep™ Human CD25 Positive Selection Cocktail contains an anti-CD25 antibody clone that recognizes epitope B of the CD25 antigen and may block some anti-CD25 antibody clones used to assess purity by flow cytometry. For purity assessment of isolated cells by flow cytometry, use the following fluorochrome-conjugated antibody clones:

- Anti-Human CD3 Antibody, Clone UCHT1 (Catalog #60011; optional), and
- Anti-human CD4 antibody, clone RPA-T4, and
- Anti-Human CD25 Antibody, Clone 2A3 (Catalog #60153), which recognizes epitope A of the CD25 antigen, and
- Anti-Human CD45 Antibody, Clone HI30 (Catalog #60018; optional), and
- Anti-human CD127 antibody, clone hIL-7R-M21, and
- Anti-human FOXP3 antibody, clone 206D

NOTE: Brilliant Violet™ antibody conjugates should be carefully titrated on EasySep™ Release-isolated cells prior to analysis by flow cytometry or fluorescence microscopy. For purity assessment with Brilliant Violet™ antibody conjugates, use of BD Horizon Brilliant™ Stain Buffer is recommended to reduce non-specific interactions. For more information, refer to the manufacturer's instructions or contact us at techsupport@stemcell.com.

Data



Starting with fresh or previously frozen PBMCs, the Treg content (CD4+CD25+FOXP3+) of the isolated fraction is typically $85.0 \pm 4.8\%$ (mean \pm SD). In the above example, the purities of the start and final isolated fractions are 1.8% and 88.2%, 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 © 2020 by STEMCELL Technologies Inc. All rights reserved including graphics and images. STEMCELL Technologies & Design, STEMCELL Shield Design, Scientists Helping Scientists, EasySep, EasyTube, RapidSpheres, RoboSep, and SepMate are trademarks of STEMCELL Technologies Canada Inc. Lymphoprep is a trademark of Alere Technologies. Brilliant Violet is a trademark of Sirigen Group Ltd. BD Horizon Brilliant is a trademark of Becton, Dickinson, and Company. 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.