# EasySep™ Human CD4+CD25+ T Cell Isolation Kit

Positive Selection

Catalog #18062

For processing 1 x 10^9 cells

STEMCELL™ TECHNOLOGIES
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 #29992 | Version 1_3_3

## Description

Isolate highly purified CD25+ cells corresponding to CD4+CD25+FOXP3+ regulatory T cells from fresh or previously frozen peripheral blood mononuclear cells (PBMCs) or leukapheresis samples using a simple, two-step procedure.

- Fast and easy-to-use
- Up to 83% purity
- No columns required

First, CD4+ T cells are pre-enriched using EasySep™ Human CD4+ T Cell Enrichment Cocktail (19052C.2) with antibodies recognizing specific cell surface markers. Then, cells expressing high levels of CD25 are selected using EasySep™ Human CD25 Positive Selection Cocktail (18231C.2), which contains an antibody recognizing the CD25 surface marker. The EasySep™ cocktails label cells with antibodies that link to magnetic particles. The cells are separated without columns using an EasySep™ magnet. Isolated cells are immediately available for downstream applications such as flow cytometry, cell culture, or DNA/RNA extraction.

## Component Descriptions

| COMPONENT NAME | COMPONENT # | QUANTITY | STORAGE | SHELF LIFE | FORMAT |
|---|---|---|---|---|---|
| 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™ D Magnetic Particles (● orange) | 19250H | 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. |
| EasySep™ Human CD25 Positive Selection Cocktail | 18231C.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 and 0.1% BSA. Includes an Fc receptor blocking antibody. |
| EasySep™ Magnetic Nanoparticles Positive Selection (● brown) | 18150H | 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™ Blocking Solution | 19710 | 6 x 1 mL | Store at 2 - 8°C or room temperature. Do not freeze. | Stable until expiry date (EXP) on label.* | A blocking solution required for further cell isolations. |

BSA - bovine serum albumin; PBS - phosphate-buffered saline; TBS - TRIS-buffered saline

* Repeated exposure to air may cause some crystallization to occur around the edge of the tube. This crystallization does not affect the performance of the blocking solution.

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 40 µm Cell Strainer (Catalog #27305) for optimal results.

After preparation, resuspend cells at 5 x 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 (LEUKO PAK)

If working with large volumes (> 150 mL), concentrate leukapheresis sample first by centrifuging at 500 x g for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original leukapheresis volume with recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of recommended medium). For small volumes (≤ 150 mL), add Ammonium Chloride Solution (Catalog #07800) directly to the leukapheresis sample.

1. Add an equal volume of Ammonium Chloride Solution to the leukapheresis sample.
2. Incubate on ice for 15 minutes.
3. Centrifuge at 500 x g for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 150 x g for 10 minutes at room temperature with the brake off. Carefully remove the supernatant.
5. Repeat step 4 one or more times until most of the platelets have been removed (indicated by a clear supernatant).
6. Resuspend the cells at 5 x 10^7 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 Table 1 for detailed instructions regarding the EasySep™ procedure.

**Table 1. EasySep™ Human CD4+CD25+ T Cell Isolation Kit Protocol**

| | | EASYSEP™ MAGNET |
|---|---|---|
| **STEP** | **INSTRUCTIONS** | **"The Big Easy" (Catalog #18001)**  |
| 1 | Prepare sample at the indicated cell concentration within the volume range. | 5 x 10^7 cells/mL<br>0.5 - 8.5 mL |
| | Add sample to required tube. | 14 mL (17 x 100 mm) polystyrene round-bottom tube<br>(e.g. Corning Catalog #352057) |
| 2 | Add T Cell Enrichment Cocktail to sample. | 50 µL/mL of sample |
| | Incubate. | RT for 10 minutes |
| 3 | Vortex D Magnetic Particles (orange).<br>NOTE: Particles should appear evenly dispersed. | 30 seconds |
| 4 | Add D Magnetic Particles (orange) to sample. | 100 µL/mL of sample<br>NOTE: Two different particles are provided in this kit. Ensure D Magnetic Particles (● orange) are used in this step. |
| | Mix and incubate. | RT for 10 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 5 mL for samples < 2 mL<br>• Top up to 10 mL for samples ≥ 2 mL |
| | Place the tube (without lid) into the magnet and incubate. | RT for 5 minutes |
| 6 | Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the pre-enriched cell suspension into a new tube. | Use a new 14 mL tube |
| 7 | Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation. | RT for 1 minute |
| 8 | Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the pre-enriched cell suspension into a new tube. | Use a new 14 mL tube |
| 9 | Add Blocking Solution to sample. | 50 µL/mL of sample (based on total volume from step 5) |
| | Mix and incubate. | RT for 15 minutes |
| 10 | Wash the cells by topping up with recommended medium and centrifuge. | 200 x g for 10 minutes at RT |
| 11 | Discard the supernatant and repeat the wash step (top up with recommended medium and centrifuge). | 200 x g for 10 minutes at RT |
| 12 | Discard the supernatant and resuspend the cell pellet to the indicated volume. | 0.5 mL |
| Continue on to CD25+ Positive Selection Protocol. | | --- |
| 13 | Add CD25 Positive Selection Cocktail to sample. | 25 µL |
| | Mix and incubate. | RT for 15 minutes |
| 14 | Mix Magnetic Nanoparticles (brown).<br>NOTE: Particles should appear evenly dispersed. | Pipette up and down more than 5 times |
| 15 | Add Magnetic Nanoparticles (brown) to sample. | 25 µL<br>NOTE: Two different particles are provided in this kit. Ensure Magnetic Nanoparticles (● brown) are used in this step. |
| | Mix and incubate. | RT for 10 minutes |
| 16 | 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 5 mL |
| | Place the tube (without lid) into the magnet and incubate. | RT for 5 minutes |
| Continue on to next page. | | Continue on to next page. |

| STEP | INSTRUCTIONS (continued) | "The Big Easy" (Catalog #18001)  |
|---|---|---|
| 17 | 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. | The supernatant, which contains CD4+ T cells, may be saved and depleted of CD25+ cells (see CD25+ Cell Depletion Protocol, Table 2) otherwise discard supernatant |
| 18 | Repeat steps as indicated. | Steps 16 and 17, three more times (total of 4 x 5-minute separations) |
| 19 | Resuspend cells in desired medium. Be sure to collect cells from the sides of the tube. | Isolated CD4+CD25+ cells are ready for use |

* 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. CD25+ Cell Depletion Protocol.** Use supernatant from step 17, Table 1.

| STEP | INSTRUCTIONS |  EasySep™ (Catalog #18000) or "The Big Easy"(Catalog #18001)  |
|---|---|---|
| 1 | Wash the cells by topping up with recommended medium and centrifuge. | 200 x g for 10 minutes at RT |
| 2 | Discard the supernatant and resuspend the cell pellet to the indicated volume. | 500 µL |
| 3 | Ensure sample is in the required tube. | • For EasySep™: 5 mL (12 x 75 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352058)<br>• For "The Big Easy": 14 mL (17 x 100 mm) polystyrene round-bottom tube (e.g. Corning Catalog #352057) |
| 4 | Add CD25 Positive Selection Cocktail to sample. | 50 µL |
| | Mix and incubate. | RT for 15 minutes |
| 5 | Mix Magnetic Nanoparticles (brown).<br>NOTE: Particles should appear evenly dispersed. | Pipette up and down more than 5 times |
| 6 | Add Magnetic Nanoparticles (brown) to sample. | 50 µL<br>NOTE: Two different particles are provided in this kit. Ensure Magnetic Nanoparticles (● brown) are used in this step. |
| | Mix and incubate. | RT for 10 minutes |
| 7 | 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 |
| | Place the tube (without lid) into the magnet and incubate. | RT for 10 minutes |
| 8 | Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the depleted cell suspension into a new tube. | Use a new tube |
| 9 | Remove the tube from the magnet and place the new tube (without lid) into the magnet and incubate for a second separation. | RT for 10 minutes |
| 10 | Pick up the magnet, and in one continuous motion invert the magnet and tube,* pouring the depleted cell suspension into a new tube. | The depleted 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 3 for detailed instructions regarding the RoboSep™ procedure.

**Table 3. RoboSep™ Human CD4+CD25+ T Cell Isolation Kit 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<br>0.5 - 8.5 mL |
| | Add sample to required tube. | 14 mL (17 x 100 mm) polystyrene round-bottom tube<br>(e.g. Corning Catalog #352057) |
| 2 | Select protocol. | • For samples < 4 mL:<br>Human CD4+ T Cell Pre-Enrichment for Regulatory T Cells 18062-small volume<br>• For samples ≥ 4 mL:<br>Human CD4+ T Cell Pre-Enrichment for Regulatory T Cells 18062-large volume |
| 3 | Vortex D Magnetic Particles (orange).<br>NOTE: Particles should appear evenly dispersed. | 30 seconds |
| 4 | Load the carousel. | Follow on-screen prompts<br>NOTE: Two different particles are provided in this kit. Ensure D Magnetic Particles (● orange) are used in this step. |
| | Start the protocol. | Press the green “Run” button |
| 5 | Unload the carousel when the run is complete. | Remove the tube containing the pre-enriched cells |
| 6 | Wash the cells by topping up with recommended medium and centrifuge. | 200 x g for 10 minutes at RT |
| | Discard the supernatant and top-up sample to indicated volume. | Top up to 10 mL |
| 7 | Transfer sample to a new tube. | 14 mL (17 x 100 mm) polystyrene round-bottom tube<br>(e.g. Corning Catalog #352057) |
| | Centrifuge. | 200 x g for 10 minutes at RT |
| 8 | Carefully remove supernatant and resuspend cell pellet in recommended medium to the indicated volume. | 0.5 mL |
| Continue on to CD25+ Positive Selection Protocol. | | --- |
| 9 | Select Protocol. | Human CD25high Positive Selection 18231-High Purity |
| 10 | Mix Magnetic Nanoparticles (brown).<br>NOTE: Particles should appear evenly dispersed. | Pipette up and down more than 5 times. |
| 11 | Load the carousel. | Follow on-screen prompts<br>NOTE: Two different particles are provided in this kit. Ensure Magnetic Nanoparticles (● brown) are used in this step. |
| | Start the protocol. | Press the green “Run” button |
| 12 | 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

ASSESSING PURITY

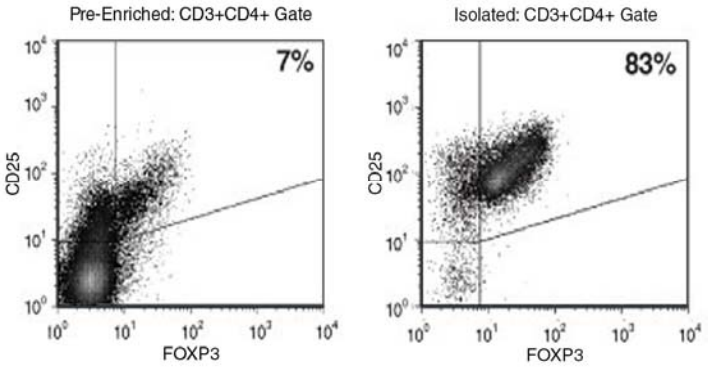
For purity assessment of CD4+ T cells by flow cytometry use the following fluorochrome-conjugated antibody clone:

- Anti-Human CD4 Antibody, Clone OKT4 (Catalog #60016)

The EasySep™ Human CD25 Positive Selection Cocktail uses an anti-CD25 antibody clone that is known to block some anti-CD25 antibody clones used to assess purity by flow cytometry. For purity assessment of CD4+CD25+ T 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 OKT4, and
- Anti-Human CD25 Antibody, Clone 2A3 (Catalog #60153), and
- Anti-human FOXP3 antibody

## Data




The CD4+CD25+ T cell content (CD4+CD25+FOXP3+) of the isolated fraction typically ranges from 61 - 83%. In the above example, the purities of the pre-enriched (after EasySep™ Human CD4+ T Cell Enrichment) and final isolated fractions are 7% and 83%, respectively.