PROCEDURE

EasySep™

POSITIVE SELECTION

V 3.2.0 STEMCELL™ TECHNOLOGIES

HUMAN PAN-CD25 POSITIVE SELECTION AND DEPLETION KIT

CATALOG #18251

THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH ROBOSEP™, THE PURPLE EASYSEP™ MAGNET OR "THE BIG EASY" SILVER EASYSEP™ MAGNET.

PRODUCT DESCRIPTION

The EasySep™ Human Pan-CD25 Positive Selection and Depletion Kit is designed to select or deplete all $CD25^+$ cells from fresh peripheral blood mononuclear cells (PBMC). For procedures on how to positively select $CD25^+$ cells, please refer to Section A (page 1) of this Product Information Sheet. For procedures on how to deplete $CD25^+$ cells, please refer to Section B (page 2). *Please note that this kit is not recommended for frozen PBMC.*

SECTION A: HUMAN PAN-CD25 POSITIVE SELECTION

FULLY AUTOMATED POSITIVE SELECTION PROTOCOL USING ROBOSEP™ (CATALOG #20000).

This procedure is used for processing **250 µL - 8.5 mL** of sample (up to 8.5 x 10^8 cells).

1. Prepare a mononuclear cell suspension at a concentration of 1 x 10^8 cells/mL in RoboSep™ Buffer (Catalog #20104) (see Notes and Tips, page 3). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep™ carousel. For samples containing 2.5 x 10^7 cells or fewer, resuspend in 250 µL.

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352057) are recommended.

2. Select the appropriate RoboSep™ protocol:

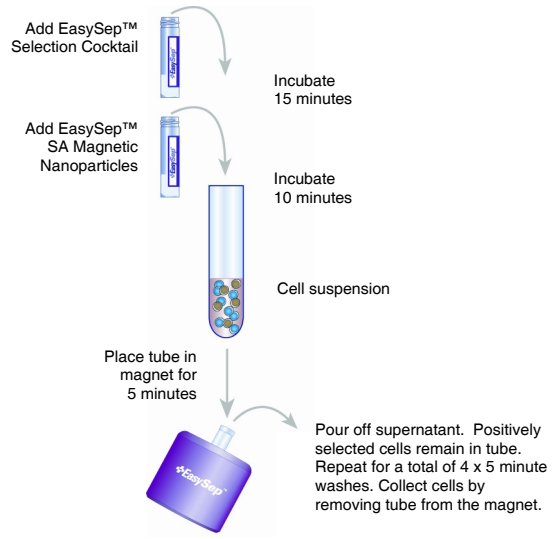
   - Human pan-CD25 Positive Selection 18251

   If a modified RoboSep™ protocol is required, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

3. Load the RoboSep™ carousel as directed by the on-screen prompts. Mix the EasySep™ SA Magnetic Nanoparticles to ensure that they are in a uniform suspension by vigorously pipetting more than 5 times. *Vortexing is not recommended.* When all desired quadrants are loaded, press the green "Run" button. All cell labeling and separation steps will be performed by RoboSep™.

4. When cell separation is complete, remove the tube containing the isolated cells from the magnet. The positively selected cells are now ready for use.

MANUAL EASYSEP™ POSITIVE SELECTION PROTOCOL DIAGRAM



MANUAL EASYSEP™ POSITIVE SELECTION PROTOCOL USING THE PURPLE EASYSEP™ MAGNET (CATALOG #18000).

This procedure is used for processing **100 µL- 2.5 mL** of sample (up to 2.5 x 10^8 cells).

1. Prepare a mononuclear cell suspension at a concentration of 1 x 10^8 cells/mL in recommended medium (see Notes and Tips, page 3). Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the Purple EasySep™ Magnet. Do not exceed a volume of 2.5 mL (i.e. 2.5 x 10^8 cells) per tube. For samples containing 10^7 cells or fewer, resuspend in 100 µL.

   Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352058) are recommended.

2. Add the EasySep™ Human Pan-CD25 Positive Selection Cocktail at **50 µL/mL cells** (e.g. for 1 mL of cells, add 50 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.

3. Mix the EasySep™ SA Magnetic Nanoparticles to ensure that they are in a uniform suspension by vigorously pipetting more than 5 times. *Vortexing is not recommended.* Add the nanoparticles at **50 µL/mL cells** (e.g. for 1 mL of cells, add 50 µL of nanoparticles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.

4. Bring the cell suspension to a **total volume** of **2.5 mL** by adding recommended medium (see Notes and Tips, page 3). Mix the cells in the tube by gently pipetting up and down 2 - 3 times. Place the tube (without cap) into the magnet. Set aside for **5 minutes**.

5. Pick up the EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant fraction. The magnetically labeled cells will remain bound inside the tube, held by the magnetic field of the EasySep™ Magnet. Leave the magnet and tube in inverted position for 2 - 3 seconds, then return to upright position. *Do not shake or blot off any drops that may remain hanging from the mouth of the tube.*

6. Remove the tube from the magnet and add **2.5 mL** of the recommended medium. Mix the cell suspension by gently pipetting up and down 2 - 3 times. Place the tube back in the magnet and set aside for **5 minutes**.

7. Repeat Steps 5 and 6 twice, and then Step 5 once more, for a total of 4 x 5-minute separations in the magnet. Remove the tube from the magnet and resuspend cells in an appropriate amount of desired medium. The positively selected cells are now ready for use.

MANUAL EASYSEP™ POSITIVE SELECTION PROTOCOL USING "THE BIG EASY" SILVER EASYSEP™ MAGNET (CATALOG #18001).

This procedure is used for processing **250 µL - 8.5 mL** of sample (up to 8.5 x 10^8 cells).

1. Prepare a mononuclear cell suspension at a concentration of 1 x 10^8 cells/mL in recommended medium (see Notes and Tips, page 3). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the Silver EasySep™ Magnet. For samples containing 2.5 x 10^7 cells or fewer, resuspend in 250 µL.

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352057) are recommended.

2. Add the EasySep™ Human Pan-CD25 Positive Selection Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.

3. Mix the EasySep™ SA Magnetic Nanoparticles to ensure that they are in a uniform suspension by vigorously pipetting more than 5 times. *Vortexing is not recommended.* Add the nanoparticles at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of nanoparticles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.

4. Bring the cell suspension to a **total volume** of **5.0 mL** (for <10^8 cells) or **10 mL** (for 1 - 8.5 x 10^8 cells) by adding recommended medium. Mix the cells in the tube by gently pipetting up and down 2 - 3 times. Place the tube (without cap) into the magnet. Set aside for **5 minutes**.

5. Pick up the EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant fraction. The magnetically labeled cells will remain bound inside the tube, held by the magnetic field of the EasySep™ Magnet. Leave the magnet and tube in inverted position for 2 - 3 seconds, then return to upright position. *Do not shake or blot off any drops that may remain hanging from the mouth of the tube.*

6. Remove the tube from the magnet and add **5.0 mL** (for <10^8 cells) or **10 mL** (for 1 - 8.5 x 10^8 cells) of the recommended medium. Mix the cell suspension by gently pipetting up and down 2 - 3 times. Place the tube back in the magnet and set aside for **5 minutes**.

7. Repeat Steps 5 and 6 twice, then Step 5 once more, for a total of 4 x 5-minute separations in the magnet. Remove the tube from the magnet and resuspend cells in an appropriate amount of desired medium. The positively selected cells are now ready for use.

SECTION B: HUMAN PAN-CD25 DEPLETION

FULLY AUTOMATED DEPLETION PROTOCOL USING ROBOSEP™ (CATALOG #20000).

This procedure is used for processing **250 µL - 8.5 mL** of sample (up to 8.5 x 10^8 cells).

1. Prepare a mononuclear cell suspension at a concentration of 1 x 10^8 cells/mL in RoboSep™ Buffer (Catalog #20104) (see Notes and Tips, page 3). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep™ carousel. For samples containing 2.5 x 10^7 cells or fewer, resuspend in 250 µL.

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352057) are recommended.

2. Select the appropriate RoboSep™ protocol:

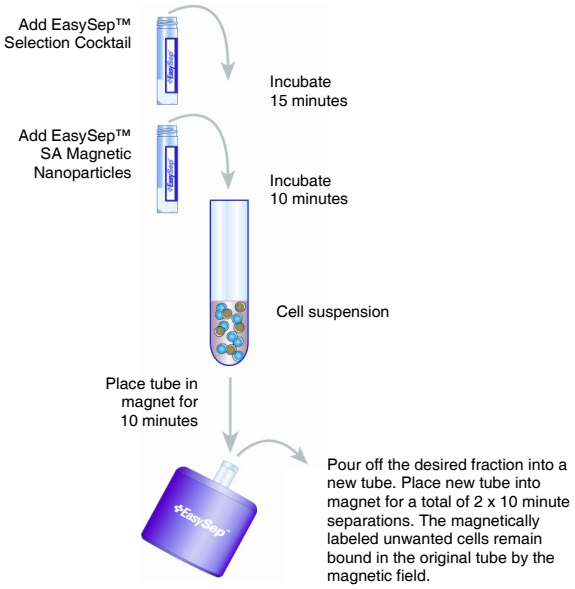
   - Human pan-CD25 Depletion 18251

   If a modified RoboSep™ protocol is required, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

3. Load the RoboSep™ carousel as directed by the on-screen prompts. Mix the EasySep™ SA Magnetic Nanoparticles to ensure that they are in a uniform suspension by vigorously pipetting more than 5 times. *Vortexing is not recommended.* When all desired quadrants are loaded, press the green "Run" button. All cell labeling and separation steps will be performed by RoboSep™.

4. When cell separation is complete, remove the depleted cells in the 50 mL tube located to the left of the tip rack. The depleted cells are now ready for use.

MANUAL EASYSEP™ DEPLETION PROTOCOL DIAGRAM



MANUAL EASYSEP™ DEPLETION PROTOCOL USING THE PURPLE EASYSEP™ MAGNET (CATALOG #18000).

This procedure is used for processing **100 µL - 2.0 mL** of sample (up to 2.0 x 10^8 cells).

1. Prepare a mononuclear cell suspension at a concentration of 1 x 10^8 cells/mL in recommended medium (see Notes and Tips, page 3). Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the Purple EasySep™ Magnet. Do not exceed a volume of 2.0 mL (i.e. 1.0 x 10^8 cells) per tube. For samples containing 10^7 cells or fewer, resuspend in 100 µL.

   Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352058) are recommended.

2. Add the EasySep™ Human Pan-CD25 Positive Selection Cocktail at **100 µL/mL cells** (e.g. for 1 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.

3. Mix the EasySep™ SA Magnetic Nanoparticles to ensure that they are in a uniform suspension by vigorously pipetting more than 5 times. *Vortexing is not recommended.* Add the nanoparticles at **50 µL/mL cells** (e.g. for 1 mL of cells, add 50 µL of nanoparticles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.

4. Bring the cell suspension to a **total volume** of **2.5 mL** by adding recommended medium. Mix the cells in the tube by gently pipetting up and down 2 - 3 times. Place the tube (without cap) into the magnet. Set aside for **10 minutes**.

5. Pick up the EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the depleted fraction into a new 5 mL polystyrene tube. The magnetically labeled unwanted cells will remain bound inside the original tube, held by the magnetic field of the EasySep™ Magnet. Leave the magnet and tube in inverted position for 2 - 3 seconds, then return to upright position. *Do not shake or blot off any drops that may remain hanging from the mouth of the tube.*

6. Remove the original tube from the magnet. Place the new tube containing the depleted cell fraction into the magnet and set aside for **10 minutes.**

7. Repeat Step 5 for a total of 2 x 10-minute separations in the magnet. The CD25 depleted cells in the new tube are now ready for use.

MANUAL EASYSEP™ DEPLETION PROTOCOL USING "THE BIG EASY" SILVER EASYSEP™ MAGNET (CATALOG #18001).

This procedure is used for processing **250 µL - 8.5 mL** of sample (up to 8.5 x 10^8 cells).

1. Prepare a mononuclear cell suspension at a concentration of 1 x 10^8 cells/mL in recommended medium (see Notes and Tips, page 3). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the Silver EasySep™ Magnet. For samples containing 2.5 x 10^7 cells or fewer, resuspend in 250 µL.

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352057) are recommended.

2. Add the EasySep™ Human Pan-CD25 Positive Selection Cocktail at **100 µL/mL cells** (e.g. for 2 mL of cells, add 200 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes.**

3. Mix the EasySep™ SA Magnetic Nanoparticles to ensure that they are in a uniform suspension by vigorously pipetting more than 5 times. *Vortexing is not recommended.* Add the nanoparticles at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of nanoparticles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes.**

4. Bring the cell suspension to a **total volume** of **5.0 mL** (for < 2.0 x10^8 cells) or **10 mL** (for 2.0 - 8.5 x 10^8 cells) by adding recommended medium. Mix the cells in the tube by gently pipetting up and down 2 - 3 times. Place the tube (without cap) into the magnet. Set aside for **10 minutes**.

5. Pick up the EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the depleted fraction into a new 14 mL polystyrene tube. The magnetically labeled unwanted cells will remain bound inside the original tube, held by the magnetic field of the EasySep™ Magnet. Leave the magnet and tube in inverted position for 2 - 3 seconds, then return to upright position. *Do not shake or blot off any drops that may remain hanging from the mouth of the tube.*

6. Remove the original tube from the magnet. Place the new tube containing the depleted cell fraction into the magnet and set aside for **10 minutes**.

7. Repeat Step 5 for a total of 2 x 10-minute separations in the magnet. The CD25 depleted cells in the new tube are now ready for use.

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V 3.2.0
#28721

CATALOG #18251

For labeling 2 x 10^9 total cells (Positive Selection)
For labeling 1 x 10^9 total cells (Depletion)

Components:

- EasySep™ Human Pan-CD25 Positive Selection Cocktail 1 mL
- EasySep™ Special Application Magnetic Nanoparticles 1 mL

EasySep™ POSITIVE SELECTION

REQUIRED EQUIPMENT:

EasySep™ Magnet (Catalog #18000), or "The Big Easy" EasySep™ Magnet (Catalog #18001), or RoboSep™ (Catalog #20000).

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep™ Human Pan-CD25 Positive Selection Cocktail and EasySep™ SA (Special Application) Magnetic Nanoparticles are designed to select or deplete all $CD25^+$ cells (cells expressing the CD25 antigen) from fresh peripheral blood mononuclear cells (PBMC).

EASYSEP™ LABELING OF HUMAN CELLS:

Target cells are specifically labeled with dextran-coated magnetic nanoparticles using bispecific Tetrameric Antibody Complexes (TAC). These complexes recognize both dextran and the target cell surface antigen (Figure 1). The small size of the magnetic dextran iron particles allows for efficient binding to the TAC-labeled cells, and does not interfere with subsequent FACS analysis. Magnetically labeled target cells are then separated from unlabeled cells using the EasySep™ procedure (pages 1 and 2).

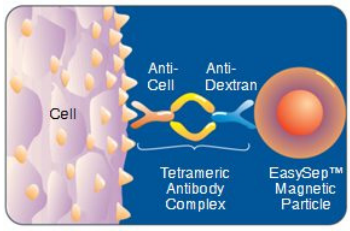


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

PREPARING A MONONUCLEAR CELL SUSPENSION. Prepare a mononuclear cell suspension from whole peripheral blood by density centrifugation.

RECOMMENDED MEDIUM. The recommended medium is RoboSep™ Buffer (Catalog #20104) or Phosphate Buffered Saline (PBS) + 2% FBS (Catalog #07905) with 1 mM EDTA. Medium should be Ca^{++} and Mg^{++} free.

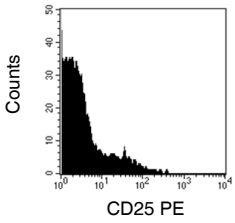
ASSESSING PURITY. The Pan-CD25 positive selection cocktail uses the anti-CD25 antibody clone MA251, which recognizes epitope B of the CD25 antigen and may block some anti-CD25 antibody clones used to assess purity by flow cytometry. We recommend using the clone 2A3 (Catalog #10512), which recognizes epitope A of the CD25 antigen, to assess purity by flow cytometry. One of the following methods can also be used to assess purity:

1. Add PE-labeled antibodies at the same time as the cocktail: Add the fluorochrome-conjugated anti-CD25 antibody at a concentration of 0.4 µg/mL immediately after adding the cocktail to provide a strong detection signal without affecting separation performance. This method labels the CD25 positive cells in the entire sample.
2. Use a secondary fluorochrome-conjugated antibody, such as FITC labeled sheep anti-mouse IgG.

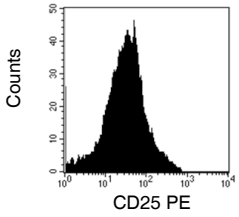
TYPICAL EASYSEP™ PAN-CD25 SELECTION AND DEPLETION PROFILES:

Positive Selection:

Start: 14.9% $CD25^+$ cells

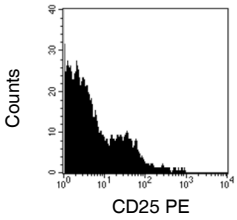


Enriched: 90.4% $CD25^+$ cells

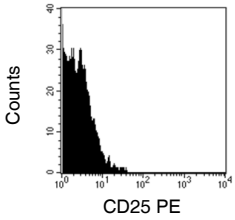


Depletion:

Start: 11.3% $CD25^+$ cells



Enriched: 0.32% $CD25^+$ cells



Starting with fresh PBMC, the $CD25^+$ cell content of the positive selection fraction typically ranges from 66 - 94% and the $CD25^+$ cell content of the depletion fraction typically ranges from 0.1 - 4.0%.

COMPONENT DESCRIPTIONS:

EASYSEP™ HUMAN PAN-CD25 POSITIVE SELECTION COCKTAIL **CODE #18251C.2**

This cocktail contains a combination of monoclonal antibodies bound in bispecific Tetrameric Antibody Complexes (TAC) which are directed against CD25 and dextran. The mouse monoclonal antibody subclass is IgG_1. This cocktail is supplied in PBS and contains an antibody against human Fc receptor. It should be noted that this product is a biological reagent, and as such cannot be completely characterized or quantified. Some variability is unavoidable.

EASYSEP™ SA MAGNETIC NANOPARTICLES **CODE #18250**

A suspension of magnetic dextran iron particles in water.

STABILITY AND STORAGE:

EASYSEP™ HUMAN PAN-CD25 POSITIVE SELECTION COCKTAIL

Product stable at 2 - 8°C until expiry date as indicated on label. Contents have been sterility tested. Do not freeze this product. This product may be shipped at room temperature (15 - 25°C), and should be refrigerated upon receipt.

EASYSEP™ SA MAGNETIC NANOPARTICLES.

Product stable at 2 - 8°C until expiry date as indicated on label. Contents have been sterility tested. Do not freeze this product. This product may be shipped at room temperature (15 - 25°C), and should be refrigerated upon receipt.

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