PROCEDURE

EasySep™

NEGATIVE SELECTION

STEMCELL TECHNOLOGIES

V 1.0.0

HUMAN MEMORY CD8⁺ T CELL ENRICHMENT KIT

CATALOG #19159

THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH ROBOSEP™ (SECTION A), THE PURPLE EASYSEP™ MAGNET (SECTION B) OR "THE BIG EASY" SILVER EASYSEP™ MAGNET (SECTION C).

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

This procedure is used for processing **1 mL - 8 mL** of sample (up to 4.0 x 10^8 cells).

1. Prepare cell suspension at a concentration of 5 x 10^7 cells/mL in RoboSep™ Buffer (Catalog #20104) (see Notes and Tips, reverse side). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep™ carousel. For sample start volumes between 0.5 - < 1.0 mL, resuspend cells to a final volume of 1.0 mL in RoboSep™ buffer.

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

2. Select the appropriate RoboSep™ protocol:
   - Human Memory CD8 T Cell Negative Selection 19159-small volume (for sample volumes between 1.0 - < 6.0 mL)
   - Human Memory CD8 T Cell Negative Selection 19159-large volume (for sample volumes between 6.0 - 8.0 mL)

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

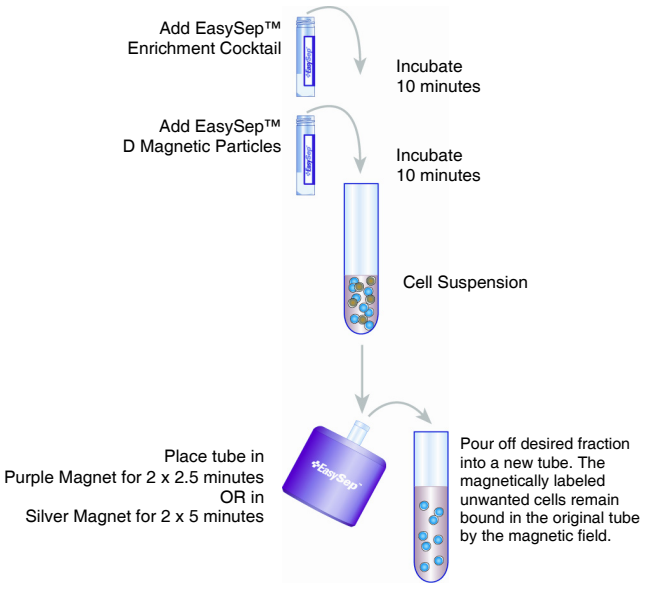
3. Load the RoboSep™ carousel as directed by the on-screen prompts. **Vortex the EasySep™ D Magnetic Particles for 30 seconds before loading. Ensure that the particles are in a uniform suspension with no visible aggregates.**

   IMPORTANT NOTE: Use of the Human Memory CD8 T Cell Negative Selection 19159-large volume protocol requires that **two** vials of EasySep™ D Magnetic Particles (Catalog #19250) be loaded onto the carousel for a single run. Place the first vial of particles in the ▲ (triangle) slot, and the second particle vial in the ● (circle) slot.

4. When all desired quadrants are loaded, press the green "Run" button. All cell labeling and separation steps will be performed by RoboSep™.

5. When cell separation is complete, remove the enriched cells in the 14 mL tube located to the left of the magnet in the second quadrant. The enriched cells are now ready for use.

MANUAL EASYSEP™ PROTOCOL DIAGRAM



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

This procedure is used for processing **100 µL - 2 mL** of sample (up to 1.0 x 10^8 cells).

1. Prepare cell suspension at a concentration of 5 x 10^7 cells/mL in recommended medium (see Notes and Tips, reverse side). Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the Purple EasySep™ Magnet.

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

2. Add the EasySep™ Human Memory CD8⁺ T Cell Enrichment 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 **10 minutes**.

3. Vortex the EasySep™ D Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

4. Add the EasySep™ D Magnetic Particles at **150 µL/mL cells** (e.g. for 2 mL of cells, add 300 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.

5. Bring the cell suspension up 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 **2.5 minutes**.

6. Pick up the EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired 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.*

7. Remove the original tube from the EasySep™ Magnet and place the new tube containing the desired cells into the magnet and set aside for **2.5 minutes**.

8. Repeat Step 6 for a total of 2 separations in the magnet (**2 x 2.5 minutes**). The negatively selected, enriched cells in the new tube are now ready for use.

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

This procedure is used for processing **250 µL - 8 mL** of sample (up to 4.0 x 10^8 cells).

1. Prepare cell suspension at a concentration of 5 x 10^7 cells/mL in recommended medium (see Notes and Tips, reverse side). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the Silver EasySep™ Magnet.

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

2. Add the EasySep™ Human Memory CD8⁺ T Cell Enrichment 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 **10 minutes**.

3. Vortex the EasySep™ D Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

4. Add the EasySep™ D Magnetic Particles at **150 µL/mL cells** (e.g. for 2 mL of cells, add 300 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.

5. Bring the cell suspension to a **total volume** of **5 mL** (for ≤ 2 x 10^8 cells) or **10 mL** (for > 2 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**.

6. Pick up the EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired 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.*

7. Remove the original tube from the EasySep™ Magnet and place the new tube containing the desired cells into the magnet and set aside for **5 minutes**.

8. Repeat Step 6 for a total of 2 separations in the magnet (**2 x 5 minutes**). The negatively selected, enriched cells in the new tube are now ready for use.

CATALOG #19159 For labeling 10^9 total cells

Components:

- EasySep™ Human Memory $CD8^+$ T Cell Enrichment Cocktail 1.0 mL
- EasySep™ D Magnetic Particles 3 x 1.0 mL

EasySep™ NEGATIVE 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 Memory $CD8^+$ T Cell Enrichment Cocktail and EasySep™ D Magnetic Particles label non-$CD8^+$ T cells and unwanted $CD8^+$ T cell subsets for magnetic separation. These reagents are designed to enrich memory $CD8^+$ T cells from fresh or previously frozen peripheral blood mononuclear cells (PBMC), buffy coat, or ammonuium chloride-lysed leukapheresis by depletion of non-memory $CD8^+$ T cells.

EASYSEP™ LABELING OF HUMAN CELLS:

Unwanted cells are specifically labeled with dextran-coated magnetic particles using bispecific Tetrameric Antibody Complexes (TAC). These complexes recognize both dextran and the cell surface antigen expressed on the unwanted cells (Figure 1). The small size of the magnetic dextran iron particles allows for efficient binding to the TAC-labeled cells. Magnetically labeled cells are then separated from unlabeled target cells using the EasySep™ procedure (reverse side).

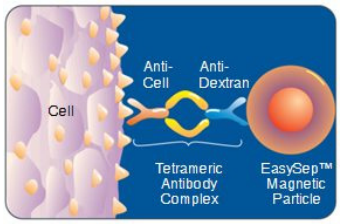


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

PREPARING THE CELL SUSPENSION

FROM WHOLE PERIPHERAL BLOOD

Prepare a mononuclear cell suspension from whole peripheral blood by density gradient centrifugation. **For previously frozen mononuclear cells, we recommend incubating the cells with DNase I (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 30 µm mesh nylon strainer for optimal results.

FROM PERIPHERAL BLOOD APHERESIS (LEUKOPAK)

If working with large volumes (>150 mL), concentrate Leukopak cells first by centrifuging at 500 x *g* for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original Leukopak volume with recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of recommended medium). For small volumes (150 mL or less), add the Ammonium Chloride Solution (Catalog #07800/07850) directly to the cell suspension.

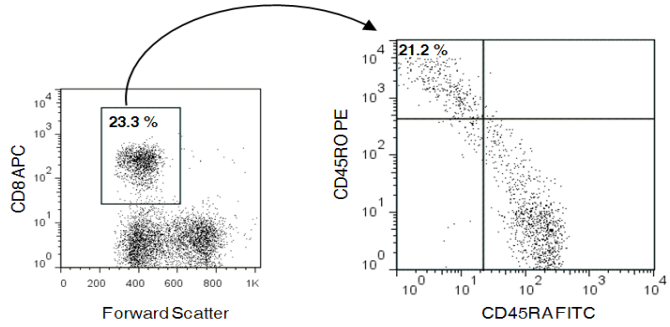
1. Add an equal volume of Ammonium Chloride Solution to the Leukopak suspension (e.g. for 5 mL of Leukopak suspension, add 5 mL Ammonium Chloride Solution).
2. Incubate 15 minutes on ice.
3. Centrifuge at 500 x *g* for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 150 x *g* for 10 minutes at room temperature (15 - 25°C) with the brake off. Carefully remove the supernatant.
5. Repeat the wash step one or more times until most of the platelets have been removed (indicated by a clear supernatant).
6. Resuspend cells at recommended cell concentration, in the recommended medium.

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.

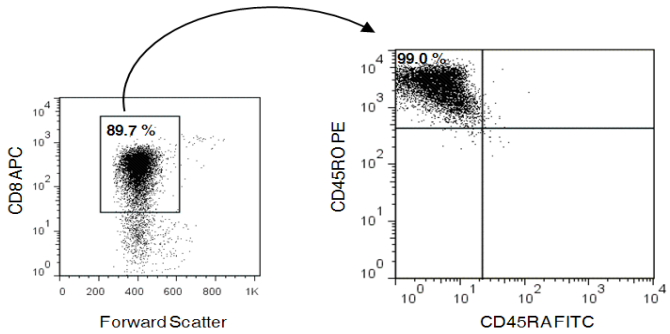
ASSESSING PURITY. Purity of memory $CD8^+$ T cells can be measured by flow cytometry after staining with fluorochrome-conjugated anti-CD8, anti-CD45RO and anti-CD45RA antibodies.

TYPICAL EASYSEP™ HUMAN MEMORY $CD8^+$ T CELL ENRICHMENT PROFILE:

Start: 4.9% $CD8^+CD45RA^-CD45RO^+$ of total peripheral blood mononuclear cells (PBMC)



Enriched: 88.8% $CD8^+CD45RA^-CD45RO^+$ of enriched fraction



Starting with mononuclear cells, the memory $CD8^+$ T cell content of the enriched fraction typically ranges from 72 - 92%.

COMPONENT DESCRIPTIONS:

EASYSEP™ HUMAN MEMORY $CD8^+$ T CELL ENRICHMENT COCKTAIL **CODE #19159C**

This cocktail contains a combination of monoclonal antibodies bound in bispecific Tetrameric Antibody Complexes (TAC) which are directed against cell surface antigens on human blood cells (CD4, CD14, CD16, CD19, CD20, CD34, CD36, CD45RA, CD56, CD61, CD66b, CD123, TCRγ/δ, glycophorin A) and dextran. The mouse monoclonal antibody subclass is IgG_1. 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™ D MAGNETIC PARTICLES **CODE #19250**

A suspension of magnetic dextran iron particles in TRIS buffer.

STABILITY AND STORAGE:

EASYSEP™ HUMAN MEMORY $CD8^+$ T CELL ENRICHMENT COCKTAIL

EASYSEP™ D MAGNETIC PARTICLES

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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