PROCEDURE

EasySep™

NEGATIVE SELECTION

STEMCELL™ TECHNOLOGIES

MOUSE NAÏVE CD8+ T CELL ISOLATION KIT

CATALOG #19858

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).

If using other EasySep™ Magnets, please visit www.stemcell.com to download the magnet-specific Product Information Sheet or contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

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

This procedure is used for processing **1.0 - 8.5 mL** of sample (up to 8.5 x 10^8 cells).

1. Prepare cell suspension at a concentration of 1 x 10^8 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 RoboSep™ carousel. Add the Normal Rat Serum (provided) at **50 µL/mL of cells** (e.g. for 2 mL of cell suspension, add 100 µL of rat serum). For sample start volumes between 0.5 - < 1 mL, resuspend cells to a final volume of 1 mL in recommended medium.

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

2. Select the appropriate RoboSep™ protocol:

   - Mouse Naive CD8+ T Cell Isolation 19858

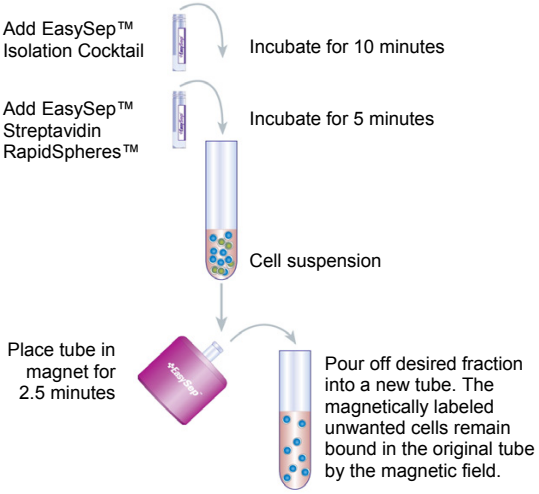
   If a modified RoboSep™ protocol is required, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

3. Vortex the EasySep™ Streptavidin RapidSpheres™ 50001 for 30 seconds before loading. Ensure that the RapidSpheres™ are in a uniform suspension with no visible aggregates.

4. Load the RoboSep™ carousel as directed by the on-screen prompts. 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 isolated cells in the 50 mL tube located to the left of the tip rack. The isolated 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 **0.25 - 2 mL** of sample (up to 2 x 10^8 cells).

1. Prepare cell suspension at a concentration of 1 x 10^8 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 EasySep™ Magnet. Add the Normal Rat Serum (provided) at **50 µL/mL of cells** (e.g. for 2 mL of cell suspension, add 100 µL of rat serum).

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

2. Add the EasySep™ Mouse Naïve CD8+ T Cell Isolation Cocktail at **50 µL/mL of 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™ Streptavidin RapidSpheres™ 50001 for 30 seconds. Ensure that the RapidSpheres™ are in a uniform suspension with no visible aggregates.

4. Add the EasySep™ Streptavidin RapidSpheres™ 50001 at **100 µL/mL of cells** (e.g. for 2 mL of cells, add 200 µL of RapidSpheres™). Mix well and incubate at room temperature (15 - 25°C) for **5 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 at room temperature (15 - 25°C) 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.* The isolated 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 **0.5 - 8.5 mL** of sample (up to 8.5 x 10^8 cells).

1. Prepare cell suspension at a concentration of 1 x 10^8 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 Big Easy" EasySep™ Magnet. Add the Normal Rat Serum (provided) at **50 µL/mL of cells** (e.g. for 2 mL of cell suspension, add 100 µL of rat serum).

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

2. Add the EasySep™ Mouse Naïve CD8+ T Cell Isolation Cocktail at **50 µL/mL of 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™ Streptavidin RapidSpheres™ 50001 for 30 seconds. Ensure that the RapidSpheres™ are in a uniform suspension with no visible aggregates.

4. Add the EasySep™ Streptavidin RapidSpheres™ 50001 at **100 µL/mL of cells** (e.g. for 2 mL of cells, add 200 µL of RapidSpheres™). Mix well and incubate at room temperature (15 - 25°C) for **5 minutes**.

5. Bring the cell suspension up to a total volume of **5 mL** (for < 4 x10^8 cells) or **10 mL** (for 4 - 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 at room temperature (15 - 25°C) 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 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.* The isolated cells in the new tube are now ready for use.

CATALOG #19858 For labeling 10^9 total cells

Components:

- EasySep™ Mouse Naïve CD8+ T Cell Isolation Cocktail 0.5 mL
- EasySep™ Streptavidin RapidSpheres™ 50001 1 mL
- Normal Rat Serum 2 mL

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REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION:

The EasySep™ Mouse Naïve CD8+ T Cell Isolation Kit is designed to isolate naïve CD8+ T cells from single cell suspensions of splenocytes or lymph nodes by negative selection. Unwanted cells are labeled with biotinylated antibodies directed against non-naïve CD8+ T cells. Labeled cells are then targeted for removal by EasySep™ Streptavidin RapidSpheres™ 50001, and separated using an EasySep™ Magnet without the use of columns. Desired cells are poured off into a new tube.

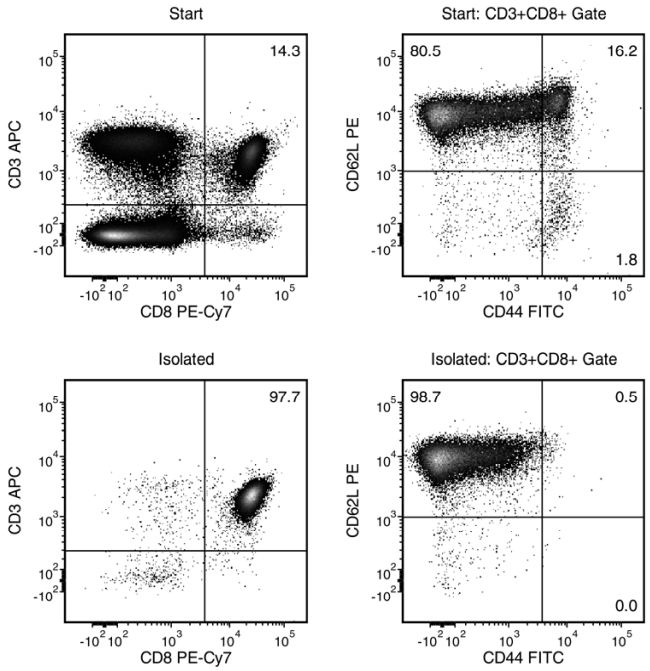
NOTES AND TIPS:

PREPARING A SINGLE CELL SUSPENSION Disrupt spleen or lymph nodes in phosphate-buffered saline (PBS) or Hank's balanced salt solution (HBSS) plus 2% fetal bovine serum (FBS). Centrifuge at 300 x *g* for 10 minutes and resuspend at 1 x 10^8 nucleated cells/mL in recommended medium. Ammonium chloride treatment is not recommended when preparing the cells for separation.

RECOMMENDED MEDIUM The recommended medium is RoboSep™ Buffer (Catalog #20104), or EasySep™ Buffer (Catalog #20144), or PBS + 2% FBS with 1 mM EDTA. HBSS can be used in place of PBS. Medium should be Ca++, Mg++, and biotin-free.

ASSESSING PURITY Purity of naïve CD8+ T cells can be measured by flow cytometry after labeling with fluorochrome-conjugated anti-CD3, anti-CD8, anti-CD44 and anti-CD62L antibodies. The anti-mouse CD44 (Ly-24) clone 5035-41.1D is not blocked by the anti-CD44 clone used in the 19858C cocktail. The 5035-41.1D clone only recognizes the Ly-24.2 isoform which is expressed by C57BL/6, C57BL/10, C57/L, C58A, AKR, 129, SJL, NZB, C3H, CE, CBA/H mouse strains. The anti-mouse/human CD44 clone IM7 is blocked by the anti-mouse CD44 antibody used in the 19858C cocktail and cannot be used to assess purity.

TYPICAL EASYSEP™ MOUSE NAÏVE CD8+ T CELL ISOLATION PROFILE:



Starting with splenocytes from an uninfected mouse, the naïve CD8+ T cell (CD3+CD8+CD44-CD62L+) content of the isolated fraction typically ranges from 92 - 98%. In the example above, the final purities of the start and isolated fractions are 11.5% and 96.4%, respectively.

COMPONENT DESCRIPTIONS:

EASYSEP™ MOUSE NAÏVE CD8+ T CELL ISOLATION COCKTAIL CODE #19858C
This cocktail contains a combination of biotinylated monoclonal antibodies directed against cell surface antigens on mouse cells of hematopoietic origin (CD4, CD11b, CD11c, CD19, CD44, CD45R, CD49b, γδTCR and TER-119). This cocktail is supplied in PBS. 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™ STREPTAVIDIN RAPIDSPHERES™ 50001 CODE #50001
A suspension of streptavidin coated magnetic particles in PBS.

NORMAL RAT SERUM CODE #13551
This normal rat serum is used to prevent non-specific binding of rat antibodies to mouse cells. Serum has been certified by the manufacturer to be mycoplasma-free.

STABILITY AND STORAGE:

EASYSEP™ MOUSE NAÏVE CD8+ T CELL ISOLATION COCKTAIL

EASYSEP™ STREPTAVIDIN RAPIDSPHERES™ 50001
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.

NORMAL RAT SERUM
Product stable at -20°C until expiry date as indicated on label. Stable for at least 2 months when stored at 2 - 8°C. Contents have been sterility tested.