PROCEDURE

EasySep™

NEGATIVE SELECTION

STEMCELL™ TECHNOLOGIES

MOUSE CD4+ T CELL ISOLATION KIT

CATALOG #19852

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's Technical Support at techsupport@stemcell.com.

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

This procedure is used for processing **0.5 - 8 mL** of sample (up to 8 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).

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

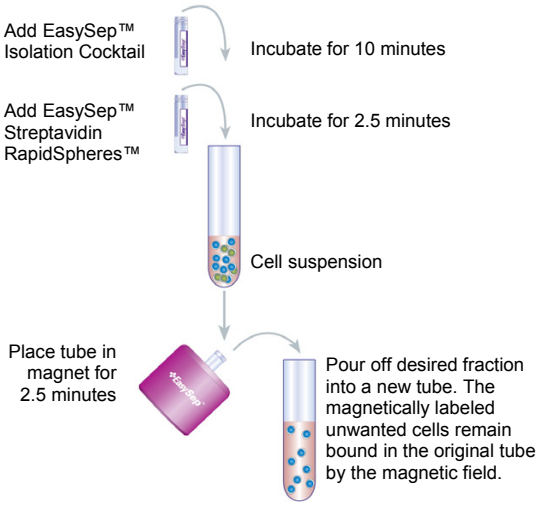
2. Select the appropriate RoboSep™ protocol:

   - Mouse CD4+ T Cell Isolation 19852

   If a modified RoboSep™ protocol is required, please contact STEMCELL Technologies's 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 CD4+ 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 **75 µL/mL of cells** (e.g. for 2 mL of cells, add 150 µL of RapidSpheres™). Mix well and incubate at room temperature (15 - 25°C) for **2.5 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 mL** of sample (up to 8 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 CD4+ 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 **75 µL/mL of cells** (e.g. for 2 mL of cells, add 150 µL of RapidSpheres™). Mix well and incubate at room temperature (15 - 25°C) for **2.5 minutes**.
5. Bring the cell suspension up to a total volume of **5 mL** (for <4 x 10^8 cells) or **10 mL** (for 4 - 8 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 #19852 For labeling 10^9 total cells

Components:

- EasySep™ Mouse CD4+ T Cell Isolation Cocktail 0.5 mL
- EasySep™ Streptavidin RapidSpheres™ 50001 1 mL
- Normal Rat Serum 2 mL

EasySep™ NEGATIVE SELECTION

REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION:

The EasySep™ Mouse CD4+ T Cell Isolation Kit is designed to isolate CD4+ T cells from single cell suspensions of splenocytes or other tissues by negative selection. Unwanted cells are labeled with biotinylated antibodies directed against non-CD4+ 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.

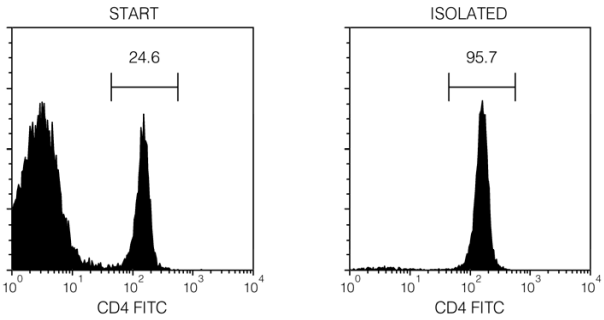
NOTES AND TIPS:

PREPARING A SINGLE CELL SUSPENSION Disrupt spleen 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 CD4+ T cells can be measured by flow cytometry after labeling with a fluorochrome-conjugated anti-CD4 antibody.

TYPICAL EASYSEP™ MOUSE CD4+ T CELL ISOLATION PROFILE:



Starting with mouse splenocytes, the CD4+ T cell content of the isolated fraction typically ranges from 89 - 96%.

COMPONENT DESCRIPTIONS:

EASYSEP™ MOUSE CD4+ T CELL ISOLATION COCKTAIL CODE #19852C
This cocktail contains a combination of biotinylated monoclonal antibodies directed against cell surface antigens on mouse cells of hematopoietic origin (CD8a, CD11b, CD11c, CD19, CD45R/B220, CD49b, TCRγ/δ and TER119). 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 CD4+ 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.

STEMCELL™ TECHNOLOGIES
TOLL-FREE T. 1 800 667 0322 • T. +1 604 877 0713 • TOLL-FREE F. 1 800 567 2899 • F. +1 604 877 0704
ORDERS@STEMCELL.COM • INFO@STEMCELL.COM • FOR FULL CONTACT DETAILS WORLDWIDE VISIT WWW.STEMCELL.COM
VERSION 1.0.0
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