

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 Technical Support at techsupport@stemcell.com.

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

This procedure is used for processing **500 µL - 8.0 mL** of sample (up to 8.0×10^8 cells).

1. Prepare nucleated cell suspension at a concentration of 1×10^8 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).

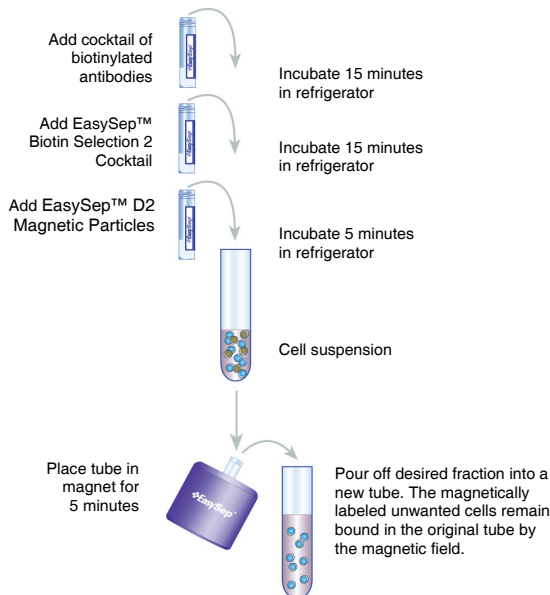
Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352057) are recommended.

2. Select the appropriate RoboSep™ protocol:
 - Mouse CD4⁺ T Cell Negative Selection 19752 (D particles)

Note: 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™ D2 Magnetic Particles for 30 seconds before loading. Ensure that the particles are in a uniform suspension with no visible aggregates.** 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 enriched cells in the 50 mL tube located to the left of the tip rack. The enriched cells are now ready for use.

MANUAL EASYSEP™ PROTOCOL DIAGRAM



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

This procedure is used for processing **250 µL - 2.0 mL** of sample (up to 2×10^8 cells).

1. Prepare cell suspension at a concentration of 1×10^8 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 Purple 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 Enrichment Cocktail at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes**.
3. Add the EasySep™ Biotin Selection Cocktail 2 at **100 µL/mL cells** (e.g. for 2 mL of cells, add 200 µL of selection cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes**.
4. Vortex the EasySep™ D2 Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
5. Add the EasySep™ D2 Magnetic Particles at **100 µL/mL cells** (e.g. for 2 mL of cells, add 200 µL of magnetic particles). Mix well and incubate in refrigerator (2 - 8°C) for **5 minutes**.
6. Bring the cell suspension up to a **total volume of 2.5 mL** by adding recommended medium without rat serum. 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**.
7. 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 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 **500 µL - 8.0 mL** of sample (up to 8.0×10^8 cells).

1. Prepare cell suspension at a concentration of 1×10^8 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 Silver 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 Enrichment Cocktail at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes**.
3. Add the EasySep™ Biotin Selection Cocktail 2 at **100 µL/mL cells** (e.g. for 2 mL of cells, add 200 µL of selection cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes**.
4. Vortex the EasySep™ D2 Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
5. Add the EasySep™ D2 Magnetic Particles at **100 µL/ mL cells** (e.g. for 2 mL of cells add 200 µL of magnetic particles). Mix well and incubate in refrigerator (2 - 8°C) for **5 minutes**.
6. Bring the cell suspension up to a **total volume of 5 mL** (for $<4 \times 10^8$ cells) or **10 mL** (for $4 - 8.0 \times 10^8$ cells) by adding recommended medium without rat serum. 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**.
7. 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 enriched cells in the new tube are now ready for use.

Components:

- EasySep™ Mouse CD4⁺ T Cell Enrichment Cocktail 0.5 mL
- EasySep™ Biotin Selection Cocktail 2 1.0 mL
- EasySep™ D2 Magnetic Particles 1.0 mL
- Normal Rat Serum 2.0 mL



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™ Mouse CD4⁺ T Cell Enrichment Cocktail, EasySep™ Biotin Selection Cocktail 2 and EasySep™ D2 Magnetic Particles label non-CD4⁺ T cells for magnetic separation. These reagents are designed to enrich CD4⁺ T cells from mouse spleen cell suspensions by depletion of non-CD4⁺ T cells.

EASYSEP™ LABELING OF MOUSE CELLS:

Unwanted cells are specifically labeled with dextran-coated magnetic particles using biotinylated antibodies against cell surface antigens expressed on the unwanted cells, and bispecific Tetrameric Antibody Complexes (TAC). These complexes recognize both dextran and biotin (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).

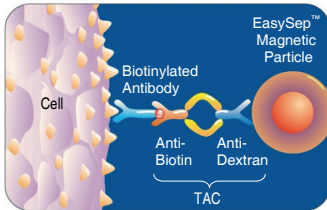


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Mouse Cells.

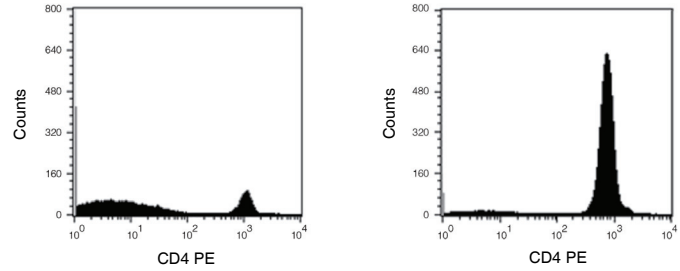
NOTES AND TIPS:

PREPARING A SINGLE CELL SUSPENSION. Disrupt spleen in Phosphate Buffered Saline (PBS) or Hank's Balanced Salt Solution plus 2% Fetal Bovine Serum (FBS). Centrifuge at $300 \times g$ for 10 minutes and resuspend at 1×10^8 nucleated cells/mL in recommended medium. Ammonium chloride treatment is not recommended when preparing the cells for separation.

OPTIMAL CELL NUMBER. The use of fewer than 5×10^7 cells per separation may result in sub-optimal performance.

RECOMMENDED MEDIUM. The recommended medium is RoboSep™ Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) + 2% FBS (Catalog #07905) with 1 mM EDTA. Hanks' Balanced Salt Solution (Hanks' BSS) (Catalog #37250) can be used in place of PBS (Catalog #37350). Medium should be Ca^{++} and Mg^{++} free.

ASSESSING PURITY. Purity of CD4⁺ T cells can be measured by flow cytometry after staining with a fluorochrome-conjugated anti-CD4 antibody (e.g. FITC anti-CD4, Catalog #10701).

TYPICAL EASYSEP™ MOUSE CD4⁺ T CELL ENRICHMENT PROFILE:Start: 22% CD4⁺ CellsEnriched: 94% CD4⁺ Cells

Starting with mouse splenocytes, the CD4⁺ cell content of the enriched fraction typically ranges from 84 - 95%.

COMPONENT DESCRIPTIONS:**EASYSEP™ MOUSE CD4⁺ T CELL ENRICHMENT COCKTAIL** CODE #19752C.3

This cocktail contains a combination of biotinylated monoclonal antibodies directed against cell surface antigens on mouse cells of hematopoietic origin (CD8, CD11b, CD11c, CD19, CD45R, CD49b, 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™ BIOTIN SELECTION COCKTAIL 2

CODE #19653

This cocktail is a combination of two mouse IgG₁ monoclonal antibodies bound in bispecific Tetrameric Antibody Complexes by rat monoclonal antibodies against mouse IgG₁. 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™ D2 MAGNETIC PARTICLES

CODE #19650

A suspension of magnetic dextran iron particles in Tris buffer.

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 ENRICHMENT COCKTAIL****EASYSEP™ BIOTIN SELECTION COCKTAIL 2****EASYSEP™ D2 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.

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.