



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 **500 µL - 8.5 mL** of sample ($\leq 8.5 \times 10^6$ cells).

1. Prepare nucleated cell suspension at a concentration of 1×10^6 cells/mL in recommended medium (see Notes and Tips, reverse side) containing 5% Normal Rat Serum (provided). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep® carousel.

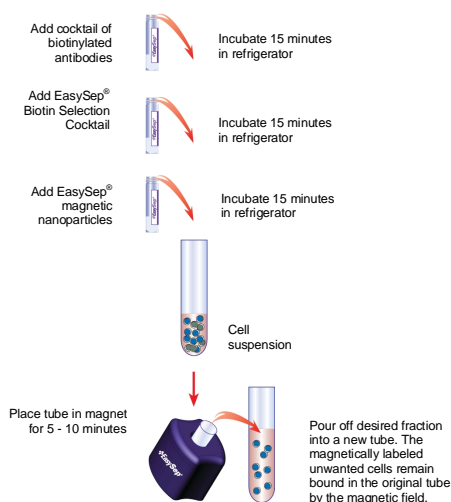
Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.

2. Select the 2-quadrant RoboSep® protocol entitled "Mouse CD4⁺ T cell Negative Selection 19752-high purity".

Note: For some applications, it may be desirable to use the 1-quadrant protocol entitled "Mouse CD4⁺ T cell Negative Selection 19752-high recovery". This may improve recovery, but will reduce purity. 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 Magnetic Nanoparticles before loading to ensure that they are in a uniform suspension by pipetting vigorously 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 enriched cells from the RoboSep® carousel:
 - After the 2-quadrant high purity protocol, collect the enriched cells in the 14 mL tube located to the left of the magnet in the second quadrant.
 - After the 1-quadrant high recovery protocol, collect the enriched cells in the 50 mL tube located to the left of the tip rack.
5. 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 ($\leq 2 \times 10^6$ cells).

1. Prepare nucleated cell suspension at a concentration of 1×10^6 cells/mL in recommended medium (see Notes and Tips, reverse side) containing 5% Normal Rat Serum (provided). 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, Catalog #352058) are recommended.
2. Add 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 Biotin Selection Cocktail 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. Mix Magnetic Nanoparticles to ensure that they are in a uniform suspension by pipetting vigorously 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 in refrigerator (2 - 8°C) for **15 minutes**.
5. Bring the cell suspension to a **total volume** of 2.5 mL by adding recommended medium without rat serum. Mix the cells in the tube by pipetting gently 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 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 the 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 containing the magnetically labeled unwanted cells from the EasySep® Magnet and place the new tube containing the desired enriched cells inside the magnet to perform a second round of magnetic separation. Set aside for 5 minutes and repeat Step 6. The enriched cells are now ready for use.

Additional Notes:

- I. For some applications it may be desirable to perform only a single round of magnetic separation and stop the procedure after completion of Step 6. This will improve recovery, but may reduce purity.
- II. Some of the desired cells may be left behind in the original tube after pouring off the desired fraction in Step 6. These cells may be recovered by resuspending the magnetically labeled cells in 2.5 mL of recommended medium and performing an additional round of magnetic separation. The supernatant of this separation step can be combined with the primary desired fraction. This will improve recovery, but may reduce purity. Purity assessment prior to pooling is therefore recommended, especially for cell populations with lower start percentages.

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

This procedure is used for processing **500 µL - 8.5 mL** of sample ($\leq 8.5 \times 10^6$ cells).

1. Prepare nucleated cell suspension at a concentration of 1×10^6 cells/mL in recommended medium (See Notes and Tips, reverse side) containing 5% Normal Rat Serum (provided). 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, Catalog #352057) are recommended.
2. Add 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 Biotin Selection Cocktail 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. Mix Magnetic Nanoparticles to ensure that they are in a uniform suspension by pipetting vigorously 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 in refrigerator (2 - 8°C) for **15 minutes**.
5. Bring the cell suspension to a **total volume** of 5 mL (for $<10^6$ cells) or 10 mL (for $1 - 8.5 \times 10^6$ cells) by adding recommended medium without rat serum. Mix the cells in the tube by pipetting gently 2 - 3 times. Place the tube (without cap) into the magnet. Set aside for **10 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 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 containing the magnetically labeled unwanted cells from the EasySep® Magnet and place the new tube containing the desired enriched cells inside the magnet to perform a second round of magnetic separation. Set aside for **10 minutes** and repeat Step 6. The enriched cells are now ready for use.

Additional Notes:

- I. For some applications it may be desirable to perform only a single round of magnetic separation and stop the procedure after completion of Step 6. This will improve recovery, but may reduce purity.
- II. Some of the desired cells may be left behind in the original tube after pouring off the desired fraction in Step 6. These cells may be recovered by resuspending the magnetically labeled cells in 2.5 mL of recommended medium and performing an additional round of magnetic separation. The supernatant of this separation step can be combined with the primary desired fraction. This will improve recovery, but may reduce purity. Purity assessment prior to pooling is therefore recommended, especially for cell populations with lower start percentages.

Components:

• EasySep [®] Negative Selection Mouse CD4 ⁺ T Cell Enrichment Cocktail	0.5 mL
• EasySep [®] Biotin Selection Cocktail	1.0 mL
• EasySep [®] Magnetic Nanoparticles	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[®] Negative Selection Mouse CD4⁺ T Cell Enrichment Cocktail, EasySep[®] Biotin Selection Cocktail and EasySep[®] Magnetic Nanoparticles 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 nanoparticles 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).

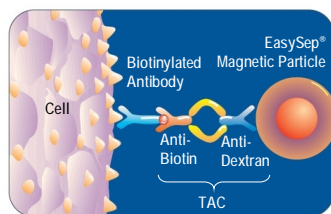


Figure 1.
Schematic Drawing of
EasySep[®] TAC Magnetic
Labeling of Mouse Cells.

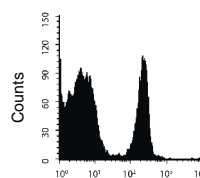
NOTES AND TIPS:

PREPARING A MONONUCLEAR CELL SUSPENSION. Disrupt spleen in 5 mL Phosphate Buffered Saline (PBS) or Hank's Balanced Salt Solution plus 2% Fetal Bovine Serum (FBS). Centrifuge and resuspend at 1×10^6 nucleated cells/mL in recommended medium. Ammonium chloride treatment is not recommended when preparing the cells for separation.

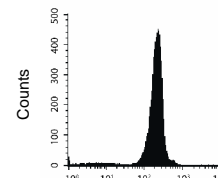
OPTIMAL CELL NUMBER. The use of fewer than 5×10^7 cells per separation may result in sub-optimal performance.

RECOMMENDED MEDIUM. The recommended medium is RoboSep[®] Buffer (Catalog #20104), or PBS + 2% FBS (Catalog #07905). Hank's Balanced Salt Solution can be used in place of PBS. 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 T CELL ENRICHMENT PROFILE:Start: 28% CD4⁺ CellsEnriched: 96% CD4⁺ Cells

CD4 FITC



CD4 FITC

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

COMPONENT DESCRIPTIONS:

EASYSep[®] NEGATIVE SELECTION MOUSE

CODE #19752C.2

CD4⁺ T CELL ENRICHMENT COCKTAIL

This cocktail contains a combination of biotinylated monoclonal antibodies purified from rat ascites fluid or hybridoma culture supernatant. The monoclonal antibodies are purified by affinity chromatography using Protein A or Protein G Sepharose. These antibodies are directed against cell surface antigens on mouse cells of hematopoietic origin (CD8, CD11b, CD19, CD45R, CD49b, TER119). This cocktail is supplied in Phosphate Buffered Saline. 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

CODE #19153

This cocktail is a combination of two mouse IgG₁ monoclonal antibodies against biotin and dextran purified from hybridoma culture supernatant. These antibodies are bound in bispecific Tetrameric Antibody Complexes by rat monoclonal antibodies against mouse IgG₁. This cocktail is supplied in Phosphate Buffered Saline. 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[®] MAGNETIC NANOPARTICLES

CODE #19150.1

A suspension of magnetic dextran iron particles in water.

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[®] NEGATIVE SELECTION MOUSE CD4⁺ T CELL ENRICHMENT 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[®] BIOTIN 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[®] 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.

NORMAL RAT SERUM

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