PROCEDURE

EasySep™ POSITIVE SELECTION

MOUSE CD4+CD62L+ T CELL ISOLATION KIT

CATALOG #18765

IMPORTANT: Two different magnetic particles are provided in the kit. It is important to follow the procedures outlined in this product information sheet carefully and use the:

- EasySep™ **Streptavidin** RapidSpheres™ 50001 only for the Mouse Naïve CD4+ T Cell Pre-Enrichment protocol
- EasySep™ **Dextran** RapidSpheres™ 50100 only for the Mouse CD4+CD62L+ T Cell Positive Selection protocol

Please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com if you have any questions.

SECTION A:

FULLY AUTOMATED PROTOCOL USING ROBOSEP™

This procedure is used for processing **0.5 - 8.5 mL** of sample (up to 8.5 x 10^8 cells).

I. RoboSep™ Mouse Naïve CD4+ T Cell Pre-Enrichment

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 (Corning®, Catalog #352057) are recommended.
2. Select the appropriate RoboSep™ protocol:
   - Mouse Naive CD4+ T Cell Pre-Enrichment 18765

   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 pre-enriched cells in the 50 mL tube located to the left of the tip rack.
6. Transfer the pre-enriched cells into a 14 mL tube and centrifuge at 200 x *g* for **10 minutes** at room temperature (15 - 25°C).

   Falcon® 14 mL Polystyrene Round-Bottom Tubes (Corning®, Catalog #352057) are recommended.
7. Carefully aspirate and discard the supernatant. Resuspend pre-enriched cells in **0.5 mL** (for 0.5 - 2 x 10^8 start cells) or **2 mL** (for > 2 - 8.5 x 10^8 start cells) of RoboSep™ Buffer (see Table 1, page 3). The sample is now ready for the CD4+CD62L+ T Cell Positive Selection protocol using RoboSep™ (Section A part II).

II. RoboSep™ Mouse CD4+CD62L+ T Cell Positive Selection

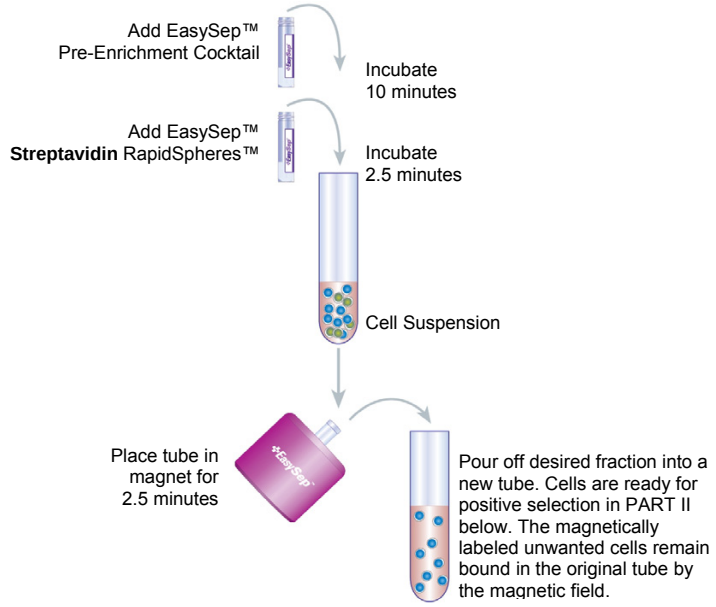
This procedure is used for processing **0.5 mL or 2 mL** of pre-enriched cells obtained in Section A part I.

1. Select the appropriate RoboSep™ protocol:
   - Mouse CD4+CD62L+ T Cell Positive Selection 18765 (18151)

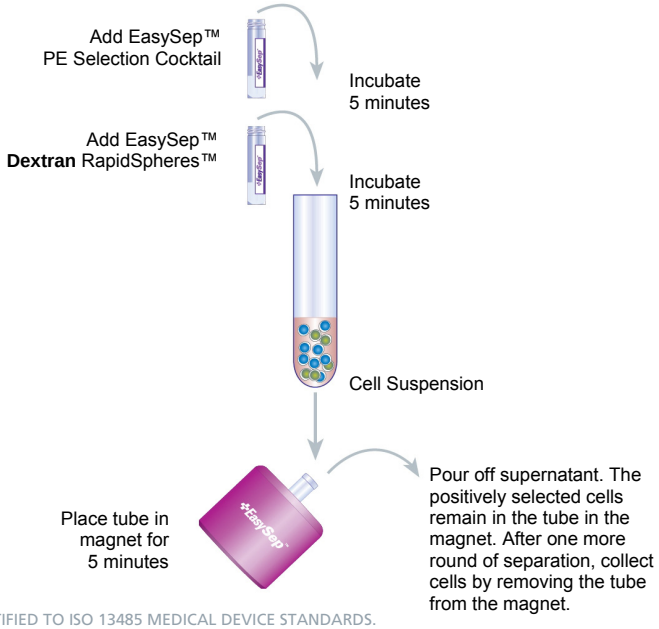
   If a modified RoboSep™ protocol is required, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.
2. Vortex the EasySep™ **Dextran** RapidSpheres™ 50100 for 30 seconds before loading. Ensure that the RapidSpheres™ are in a uniform suspension with no visible aggregates.
3. 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™.
4. When cell separation is complete, remove the tube containing the isolated cells from the magnet. Resuspend in an appropriate amount of medium. The isolated cells are now ready for use.

MANUAL EASYSEP™ PROTOCOL DIAGRAM

PART I: NAÏVE CD4+ T CELL PRE-ENRICHMENT



PART II: CD4+CD62L+ T CELL POSITIVE SELECTION



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SECTION B:

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

I. EasySep™ Mouse Naïve CD4+ T Cell Pre-Enrichment

This procedure is used for processing **0.1 - 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 (Corning®, Catalog #352058) are recommended.
2. Add the EasySep™ Mouse Naïve CD4+ T Cell Pre-Enrichment 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.*
7. Centrifuge the pre-enriched cells in the new tube at 200 x *g* for **10 minutes** at room temperature (15 - 25°C). Carefully aspirate and discard the supernatant. Resuspend pre-enriched cells in **100 µL** (for 0.1 - 0.3 x 10^8 start cells) or **250 µL** (for > 0.3 - 2 x 10^8 start cells) of the recommended medium (see Table 1, page 3). The sample is now ready for the CD4+CD62L+ T Cell Positive Selection protocol (Section B part II, see below).

II. EasySep™ Mouse CD4+CD62L+ T Cell Positive Selection

This procedure is used for processing **100 µL or 250 µL** of pre-enriched cells obtained in Section B part I (see above).

1. Add the EasySep™ PE Selection Cocktail at **40 µL/mL of pre-enriched cells** (e.g. for 100 µL of pre-enriched cells, add 4 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **5 minutes**.
2. Vortex the EasySep™ **Dextran** RapidSpheres™ 50100 for 30 seconds. Ensure that the RapidSpheres™ are in a uniform suspension with no visible aggregates.
3. Add the EasySep™ **Dextran** RapidSpheres™ 50100 at **25 µL/mL of pre-enriched cells** (e.g. for 100 µL of pre-enriched cells, add 2.5 µL of RapidSpheres™). Mix well and incubate at room temperature (15 - 25°C) for **5 minutes**.
4. 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 **5 minutes**.
5. Pick up the EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant fraction. The magnetically labeled 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.*
6. Remove the tube from the magnet and add **2.5 mL** of recommended medium. Mix the cell suspension by pipetting up and down 2 - 3 times. Place the tube back in the magnet and set aside for **5 minutes**.
7. Repeat step 5, for a total of 2 x 5-minute separations in the magnet. Remove the tube from the magnet and resuspend cells in an appropriate amount of desired medium. The isolated cells are now ready for use.

SECTION C:

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

I. EasySep™ Mouse Naïve CD4+ T Cell Pre-Enrichment

This procedure is used for processing **0.25 - 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 (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® 14 mL Polystyrene Round-Bottom Tubes (Corning®, Catalog #352057) are recommended.
2. Add the EasySep™ Mouse Naïve CD4+ T Cell Pre-Enrichment 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 start cells) or **10 mL** (for 4 - 8.5 x 10^8 start 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.*
7. Centrifuge the pre-enriched cells in the new tube at 200 x *g* for **10 minutes** at room temperature (15 - 25°C). Carefully aspirate and discard the supernatant. Resuspend pre-enriched cells in **0.5 mL** (for 0.25 - 2 x 10^8 start cells) or **2 mL** (for > 2 - 8.5 x 10^8 start cells) of the recommended medium (see Table 1, page 3). The sample is now ready for the CD4+CD62L+ T Cell Positive Selection protocol (Section C part II, see below).

II. EasySep™ Mouse CD4+CD62L+ T Cell Positive Selection

This procedure is used for processing **0.5 mL or 2 mL** of pre-enriched cells obtained in Section C part I (see above).

1. Add the EasySep™ PE Selection Cocktail at **40 µL/mL of pre-enriched cells** (e.g. for 0.5 mL of pre-enriched cells, add 20 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **5 minutes**.
2. Vortex the EasySep™ **Dextran** RapidSpheres™ 50100 for 30 seconds. Ensure that the RapidSpheres™ are in a uniform suspension with no visible aggregates.
3. Add the EasySep™ **Dextran** RapidSpheres™ 50100 at **25 µL/mL of pre-enriched cells** (e.g. for 0.5 mL of pre-enriched cells, add 12.5 µL of RapidSpheres™). Mix well and incubate at room temperature (15 - 25°C) for **5 minutes**.
4. Bring the cell suspension up to a total volume of **5 mL** (for < 4 x 10^8 start cells) or **10 mL** (for 4 - 8.5 x 10^8 start 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 **5 minutes**.
5. Pick up the EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant fraction. The magnetically labeled 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.*
6. Remove the tube from the magnet and add **5 mL** (for < 4 x 10^8 start cells) or **10 mL** (for 4 - 8.5 x 10^8 start cells) of recommended medium. Mix the cell suspension by pipetting up and down 2 - 3 times. Place the tube back in the magnet and set aside for **5 minutes**.
7. Repeat step 5, for a total of 2 x 5-minute separations in the magnet. Remove the tube from the magnet and resuspend cells in an appropriate amount of desired medium. The isolated cells are now ready for use.

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REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION AND APPLICATIONS:

The EasySep™ Mouse CD4+CD62L+ T Cell Isolation Kit is designed to isolate naïve CD4+ T cells from single-cell suspensions of splenocytes or other tissues by a two-step method. Naïve CD4+ T cells are first pre-enriched by negative selection using the EasySep™ Mouse Naïve CD4+ T Cell Pre-Enrichment Cocktail. Unwanted cells are targeted for removal with biotinylated antibodies directed against non-naïve CD4+ T cells (CD8, CD11b, CD11c, CD19, CD24, CD25, CD45R, CD49b, TCRγ/δ, TER119) and streptavidin-coated magnetic particles (Streptavidin RapidSpheres™). Labeled cells are separated using an EasySep™ magnet without the use of columns. Desired cells are poured off into a new tube. Following pre-enrichment, CD4+CD62L+ cells, pre-labeled with CD62L PE, are isolated using the EasySep™ PE selection cocktail and dextran-coated magnetic particles (Dextran RapidSpheres™). Labeled cells are separated using an EasySep™ magnet and remain in the tube while the unwanted cells are poured off.

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). Remove clumps and debris by passing the cell suspension through a 70 µm mesh nylon strainer. 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.

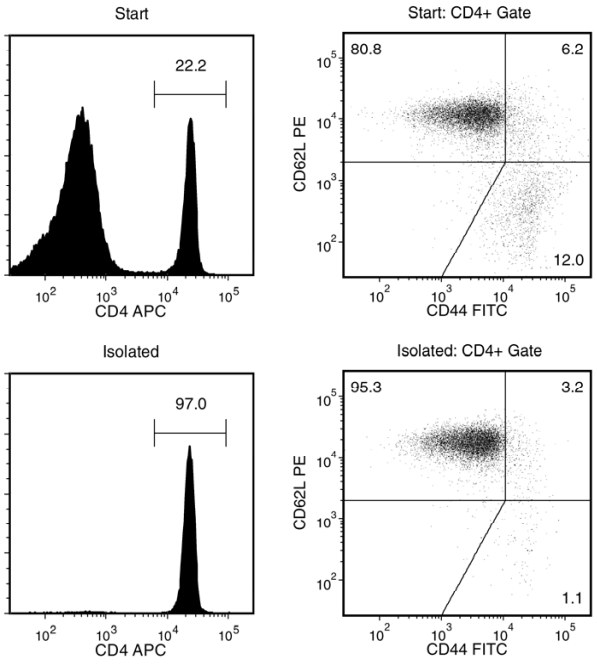
TABLE 1. Recommended Resuspension Volumes for CD4+CD62L+ T Cell Positive Selection Protocol

MAGNET	NAÏVE CD4+ T CELL PRE-ENRICHMENT		CD4+CD62L+ T CELL POSITIVE SELECTION
	STARTING CELL NUMBER	VOLUME	RESUSPENSION VOLUME
Purple	≤ 0.3 x 10^8	0.1 - 0.3 mL	100 µL
	> 0.3 - 2 x 10^8	> 0.3 - 2 mL	250 µL
Silver	0.25 - 2 x 10^8	0.25 - 2 mL	0.5 mL
	> 2 - 8.5 x 10^8	> 2 - 8.5 mL	2 mL
RoboSep™	0.5 - 2 x 10^8	0.5 - 2 mL	0.5 mL
	> 2 - 8.5 x 10^8	> 2 - 8.5 mL	2 mL

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

ASSESSING PURITY Purity of CD4+CD62L+ T cells can be measured by flow cytometry after labeling with fluorochrome-conjugated anti-CD4 (e.g. Anti-Mouse CD4 Antibody, Clone RM4-5, Catalog #60017) and anti-CD44 (e.g. Anti-Mouse CD44 Antibody, Clone IM7, Catalog #60068) antibodies; the positively selected CD62L+ cells have already been PE-labeled.

TYPICAL EASYSEP™ MOUSE CD4+CD62L+ T CELL ISOLATION PROFILE:



Starting with a single-cell suspension of splenocytes, the naïve CD4+ T cell (CD4+CD44^{low} CD62L^{high}) content of the final isolated fraction typically ranges from 91.7% - 96.7%. In the example above, the final purities of the start and isolated fractions are 17.9% and 92.4%, respectively.

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CATALOG #18765 For labeling up to 10^9 total cells

Components:

- EasySep™ Mouse Naïve CD4+ T Cell Pre-Enrichment Cocktail 0.5 mL
- EasySep™ Streptavidin RapidSpheres™ 50001 1 mL
- EasySep™ PE Selection Cocktail 1 mL
- EasySep™ Dextran RapidSpheres™ 50100 1 mL
- Normal Rat Serum 2 mL

COMPONENT DESCRIPTIONS:

EASYSEP™ MOUSE NAÏVE CD4+ T CELL PRE-ENRICHMENT COCKTAIL

CODE #18765C

This cocktail contains a combination of biotinylated monoclonal antibodies directed against cell surface antigens on mouse cells of hematopoietic origin (CD8, CD11b, CD11c, CD19, CD24, CD25, CD45R, CD49b, TCRγ/δ, TER119) and a PE-conjugated CD62L antibody. This cocktail is supplied in PBS with 0.1% bovine serum albumin (BSA). 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.

EASYSEP™ PE SELECTION COCKTAIL **CODE #18151**

This cocktail contains a combination of monoclonal antibodies purified from hybridoma culture supernatant by affinity chromatography using Protein A or Protein G Sepharose. These antibodies are bound in antibody complexes which are directed against phycoerythrin and dextran. The mouse monoclonal antibody subclass is 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™ DEXTRAN RAPIDSPHERES™ 50100 **CODE # 50100**

A suspension of dextran-coated magnetic 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™ MOUSE NAÏVE CD4+ T CELL PRE-ENRICHMENT COCKTAIL
EASYSEP™ STREPTAVIDIN RAPIDSPHERES™ 50001
EASYSEP™ PE SELECTION COCKTAIL
EASYSEP™ DEXTRAN RAPIDSPHERES™ 50100

Products stable at 2 - 8°C until expiry date as indicated on label. Contents have been sterility tested. Do not freeze these products. These products 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.