

**PROCEDURE** **Positive Selection**

**+EasySep®**

**Biotin Selection Kit**

**And More!**

**CATALOG #18553, #18556, #18559**

Version 4.1.1

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 **250 µL - 8.5 mL** of sample (up to  $1.7 \times 10^9$  cells).

1. Prepare single cell suspension at a concentration of  $1 \times 10^8$  cells/mL in RoboSep® Buffer (Catalog #20104). For rare cells, start with a concentration of  $2 \times 10^8$  cells/mL (see Notes and Tips, reverse side). For samples containing  $2.5 \times 10^7$  cells or fewer, resuspend in 250 µL. 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 (Becton Dickinson, Catalog #352057) are recommended.*

2. Add species-specific FcR blocking antibody at 100 µL/mL for human cells (supplied with Catalog #18553) or 10 µL/mL for mouse cells (supplied with Catalog #18556) and mix well. See Product Description and Applications (reverse side) for other species (Catalog #18559).

3. Select the appropriate RoboSep® protocol:

- For most human samples, select the protocol entitled "Human Biotin Positive Selection 18553-base".
- For most mouse samples, select the protocol entitled "Mouse Biotin Positive Selection 18556-base".
- For most other samples, select the protocol entitled "Any Species Biotin Positive Selection 18559-base".

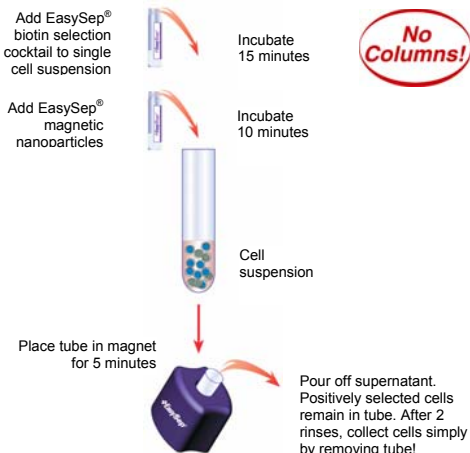
Some modifications to the base RoboSep® protocol may be required for optimal results (see Notes and Tips). Please consult the RoboSep® user manual, or contact StemCell Technologies' Technical Support at techsupport@stemcell.com.

4. Transfer primary biotinylated antibody\* to the empty vial provided at a concentration of 6 - 60 µg/mL (for a final concentration of 0.3 - 3.0 µg/mL when using the base protocols above). The minimum volume required will be stated on the RoboSep® screen.

**Note:** Titrate biotinylated antibody for optimal purity and recovery. Cell recovery increases with increased labeling. However, excess antibody can reduce purity.

5. Load the RoboSep® carousel as directed by the on-screen prompts. Mix EasySep® Magnetic Nanoparticles before loading to ensure that they are in a uniform suspension by pipetting up and down vigorously more than 5 times. When all desired quadrants are loaded, press the green "Run" button. All cell labeling and separation steps will be performed by RoboSep®.

6. When cell separation is complete, remove the tube containing the isolated cells from the magnet. The positively selected cells are now ready for use.



**B) Manual EasySep® Protocol Using Purple EasySep® Magnet (Catalog #18000).**

This procedure is used for processing **100 µL - 2.5 mL** of sample (up to  $5 \times 10^8$  cells). Some modifications to this protocol may be required (see Notes and Tips, reverse side).

1. Prepare single cell suspension at a concentration of  $1 \times 10^8$  cells/mL in recommended medium. For rare cells, start with a concentration of  $2 \times 10^8$  cells/mL (see Notes and Tips). For samples containing  $10^7$  cells or fewer, resuspend in 100 µL. Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the EasySep® magnet.

*Falcon™ 5 mL Polystyrene Round-Bottom Tubes (Becton Dickinson, Catalog #352058) are recommended.*

2. Add species-specific FcR blocking antibody at 100 µL/mL for human cells (supplied with Catalog #18553) or 10 µL/mL for mouse cells (supplied with Catalog #18556) and mix well. See Product Description and Applications (reverse side) for other species (Catalog #18559).

3. Add primary biotinylated antibody\* at a final concentration of **0.3 - 3.0 µg/mL**. Mix well and incubate at room temperature for **15 minutes**.

**Note:** Titrate biotinylated antibody for optimal purity and recovery. Cell recovery increases with increased labeling. However, excess antibody can reduce purity.

4. Add EasySep® Biotin Selection Cocktail at **100 µL/mL** cells (e.g. for 2 mL of cells, add 200 µL of cocktail). Mix well and incubate at room temperature for **15 minutes**.

5. Mix EasySep® Magnetic Nanoparticles to ensure that they are in a uniform suspension by vigorously pipetting up and down more than 5 times. Vortexing is not recommended. Add the particles at **50 µL/mL** cells (e.g. for 2 mL of cells, add 100 µL of nanoparticles). Mix well and incubate at room temperature for **10 minutes**.

6. Bring the cell suspension 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 for **5 minutes** (see Notes and Tips - Optimizing Recovery)

7. Pick up the magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant fraction. The magnetically labeled cells will remain inside the 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.*

8. Remove the tube from the magnet and add 2.5 mL recommended medium. Mix the cells by gently pipetting up and down 2 - 3 times. Place the tube back in the magnet and set aside for **5 minutes**.

9. Repeat Steps 7 and 8, and then Step 7 once more, for a total of 3 x 5-minute separations in the magnet. (For mouse separations, or low-frequency cell types, a 4<sup>th</sup> round of separation is recommended for higher purity; see Notes and Tips). Remove the tube from the magnet and resuspend cells in an appropriate amount of desired medium. The positively selected cells are now ready for use.

**C) Manual EasySep® Protocol Using "The Big Easy" Silver EasySep® Magnet (Catalog #18001).**

This procedure is used for processing **250 µL - 8 mL** of sample (up to  $1.6 \times 10^9$  cells). Some modifications to this protocol may be required (see Notes and Tips, reverse side).

1. Prepare single cell suspension at a concentration of  $1 \times 10^8$  cells/mL in recommended medium. For rare cells, start with a concentration of  $2 \times 10^8$  cells/mL (see Notes and Tips). For samples containing  $2.5 \times 10^7$  cells or fewer, resuspend in 250 µL. Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the silver magnet.

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

2. Add species-specific FcR blocking antibody at 100 µL/mL for human cells (supplied with Catalog #18553) or 10 µL/mL for mouse cells (supplied with Catalog #18556) and mix well. See Product Description and Applications (reverse side) for other species (Catalog #18559).

3. Add primary biotinylated antibody\* at a final concentration of **0.3 - 3.0 µg/mL**. Mix well and incubate at room temperature for **15 minutes**.

**Note:** Titrate biotinylated antibody for optimal purity and recovery. Cell recovery increases with increased labeling. However, excess antibody can reduce purity.

4. Add EasySep® Biotin Selection Cocktail at **100 µL/mL** cells (e.g. for 2 mL of cells, add 200 µL of cocktail). Mix well and incubate at room temperature for **15 minutes**.

5. Mix EasySep® Magnetic Nanoparticles to ensure that they are in a uniform suspension by pipetting up and down vigorously more than 5 times. Vortexing is not recommended. Add the particles at **50 µL/mL** cells (e.g. for 2 mL of cells, add 100 µL of nanoparticles). Mix well and incubate at room temperature for **10 minutes**.

6. Bring the cell suspension to a **total volume** of 5.0 mL (for  $<10^8$  cells) or 10 mL (for  $1 \times 10^8 - 1.6 \times 10^9$  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 for **5 minutes** (see Notes and Tips - Optimizing Recovery).

7. 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 inside the tube, held by the magnetic field of the EasySep® Magnet. Leave the magnet and tube in the 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.*

8. Remove the tube from the magnet and add 5.0 mL (for  $<10^8$  cells) or 10 mL (for  $1 \times 10^8 - 1.6 \times 10^9$  cells) recommended medium. Mix the cells by gently pipetting up and down 2 - 3 times. Place the tube back in the magnet and set aside for **5 minutes**.

9. Repeat Steps 7 and 8, then Step 7 once more, for a total of 3 x 5-minute separations in the magnet. (For mouse separations, or low-frequency cell types, a 4<sup>th</sup> round of separation is recommended for higher purity; see Notes and Tips). Remove the tube from the magnet and resuspend cells in an appropriate amount of desired medium. The positively selected cells are now ready for use.

\*Users of this selection kit should ensure that they are entitled to use the antibody of interest. StemCell Technologies is not responsible for patent infringements or violations that may occur when using this product.

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October 2007

**FOR RESEARCH USE ONLY**

**#28513**

<b>Catalog #18553 (Human)</b>	For labeling 10 <sup>9</sup> total cells
<b>Catalog #18556 (Mouse)</b>	For labeling 10 <sup>9</sup> total cells
<b>Catalog #18559 (Other)</b>	For labeling 10 <sup>9</sup> total cells
<b>Components:</b>	
• EasySep <sup>®</sup> Biotin Selection Cocktail	1.0 mL
• Species-Specific Blocker (Human & Mouse Only):	
Anti-Human CD32 (Fcγ RII) Blocker (#18553 Only)	1.0 mL
Mouse FcR Blocker (#18556 Only)	0.1 mL
• EasySep <sup>®</sup> Magnetic Nanoparticles	1.0 mL
• RoboSep <sup>®</sup> Vial for Primary Conjugated Antibody (not required for manual use)	1 vial

**REQUIRED EQUIPMENT:**

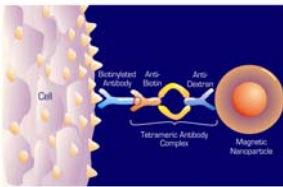
EasySep<sup>®</sup> Magnet (Catalog #18000), or “The Big Easy” EasySep<sup>®</sup> Magnet (Catalog #18001), or RoboSep<sup>®</sup> (Catalog #20000).

**PRODUCT DESCRIPTION AND APPLICATIONS:**

EasySep<sup>®</sup> Biotin Selection Cocktail and EasySep<sup>®</sup> Magnetic Nanoparticles target cells labeled with biotinylated antibodies (not supplied) for magnetic positive selection. The species-specific FcR blocker (anti-human CD32, anti-mouse CD16/32) is used to prevent non-specific selection of monocytes and macrophages. *Note: When selecting cells from other species, an appropriate species-specific FcR blocking antibody may be required to achieve desired purities. A final concentration of 0.5 - 3.0 µg/mL is recommended for the blocking antibody.*

**EASYSEP<sup>®</sup> LABELING OF CELLS:**

Target cells that have been specifically labeled with biotinylated antibody are then labeled with dextran-coated magnetic nanoparticles using bispecific Tetrameric Antibody Complexes (TAC). These complexes recognize both dextran and the biotin molecule on the biotinylated antibody (Figure 1). Magnetically labeled cells are then separated from unlabeled cells using the EasySep<sup>®</sup> procedure (reverse side).



**Figure 1.**  
Schematic Drawing of EasySep<sup>®</sup> TAC Magnetic Labeling of Human Cells.

**NOTES AND TIPS:**

**Preparing a Single Cell Suspension.** Prepare a mononuclear cell suspension from human peripheral blood by Ficoll-Paque™ PLUS density separation (Catalog #07957). Previously frozen mononuclear cells should be incubated with 100 µg/mL DNase I (Catalog #07900) in medium without EDTA for at least 15 minutes at room temperature prior to labeling and separation to reduce clumping. Filter clumpy suspensions through a 70 µm mesh nylon strainer. Mouse spleen and bone marrow cells should be prepared following standard procedures and filtered through a 70 µm mesh nylon strainer. Any other sample in single cell suspension can also be used.

**Recommended Medium.** The recommended medium is Phosphate Buffered Saline (PBS) with 2% Fetal Bovine Serum (FBS) (Catalog #07905) and 1 mM EDTA. Medium should be Ca<sup>++</sup> and Mg<sup>++</sup> free.

**Assessing Purity.** The purity of the positively selected cells can be assessed by flow cytometry using one of the following methods:

1. Add fluorochrome-conjugated antibody to the selected cells. **Note: The biotinylated antibody may block the staining antibody.**
2. Add fluorochrome-conjugated antibody at the same time as the biotinylated antibody but at 1/10 to 1/5 the concentration (for an example, see human CD3 selection profile).
3. Use fluorochrome-conjugated antibodies to alternative cell surface markers (for an example, see mouse CD4 selection profile).
4. Use a secondary fluorochrome-conjugated antibody, such as FITC-labeled sheep anti-mouse IgG.

**Optimizing Purity.** For samples with a desired cell starting frequency of less than 10 - 15%, additional separation rounds will likely improve purity. If desired, repeat Steps 7 and 8 an additional 1 - 3 times. Please note that recovery will decrease with each additional round of separation. When isolating cells representing less than 2% of the initial population, the purity of the enriched sample may be improved by starting with a cell concentration of 2 x 10<sup>9</sup> cells/mL. For some cell types, decreasing the amount of Biotin Selection Cocktail added can increase purity while decreasing recovery. This will also reduce the side scatter observed during subsequent flow cytometry analysis. Performance may also be improved by adding a wash step. After incubating the sample with biotinylated antibody (Step 3), wash once with 10-fold excess medium and resuspend to original volume. Please note that this wash step can not be automated by RoboSep<sup>®</sup>.

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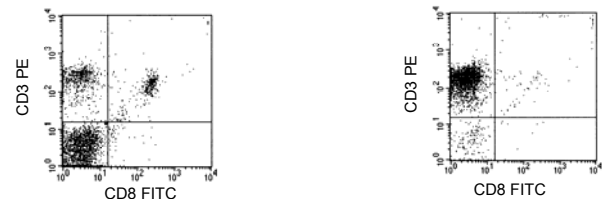
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**Optimizing Recovery.** Recovery of positively selected biotin-labeled cells is dependent on the quality of the biotinylated antibody used. Antibodies that have expired or that have been stored improperly may show lower affinity for the surface marker on the target cell, resulting in lower recovery. It is important to add sufficient biotinylated antibody to ensure a high purity and recovery. We recommend that the concentration of the biotinylated antibody be between 0.3 - 3 µg/mL. It is recommended to titrate the biotinylated antibody to achieve optimal purity and recovery. Recovery may also be improved by increasing separation times in the magnet from 5 minutes to 10 minutes for each round of separation.

**EXAMPLE: EASYSEP<sup>®</sup> BIOTIN SELECTION PROFILE - HUMAN CD3**  
Start: 60% CD3<sup>+</sup> Cells      Selected: 97.4% CD3<sup>+</sup> Cells



**EXAMPLE: EASYSEP<sup>®</sup> BIOTIN SELECTION PROFILE - MOUSE CD4**  
Start: 22.8% CD4<sup>+</sup> Cells (CD3<sup>+</sup>CD8<sup>-</sup>)      Selected: 93.9% CD4<sup>+</sup> Cells (CD3<sup>+</sup>CD8<sup>-</sup>)



**COMPONENT DESCRIPTIONS:**

**EasySep<sup>®</sup> Biotin Selection Cocktail** **code #18153**  
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 bispecific tetrameric antibody complexes (TAC) which are directed against biotin and dextran. These mouse monoclonal antibodies are of subclass IgG<sub>1</sub>. 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.

**Anti-Human CD32 (Fcγ RII) Blocker** **code #18520**  
This antibody recognizes human CD32 (Fcγ RII) present on monocytes, platelets, macrophages, and granulocytes. Supplied in PBS.

**EasySep<sup>®</sup> Mouse FcR Blocker** **code #18720**  
This antibody recognizes mouse CD16/CD32 (Fcγ III/II Receptor) present on monocytes, macrophages and other FcR<sup>+</sup> cells. Supplied in PBS in 0.1% Bovine Serum Albumin (BSA) and 0.1% sodium azide.

**EasySep<sup>®</sup> Magnetic Nanoparticles** **code #18150**  
A suspension of magnetic dextran iron particles in water.

**RoboSep<sup>®</sup> Vial for Primary Conjugated Antibody** **code #18550**  
Empty vial compatible with RoboSep<sup>®</sup> - the fully automated cell separator. If using RoboSep<sup>®</sup>, transfer biotinylated antibody to this empty vial before loading into the appropriate position in the RoboSep<sup>®</sup> carousel. If not using RoboSep<sup>®</sup>, this vial can be discarded.

**STABILITY AND STORAGE:**

**EasySep<sup>®</sup> Biotin Selection Cocktail**  
Stable at 4°C for 2 years. Do not freeze this product. Contents sterile in unopened tube. This product may be shipped at room temperature, and should be refrigerated upon receipt.

**Human or Mouse FcR Blocker**  
Stable at 4°C for 2 years (human) or 1 year (mouse). Contents sterile in unopened tube. This product may be shipped at room temperature, and should be refrigerated upon receipt. Do not freeze.

**EasySep<sup>®</sup> Magnetic Nanoparticles**  
Stable at 4°C for 2 years. Contents sterile in unopened tube. This product may be shipped at room temperature, and should be refrigerated upon receipt.

**Hazardous Ingredient: Sodium Azide.** Avoid exposure to skin and eyes, ingestion and contact with heat, acids and metals. Wash exposed skin with soap and water. Flush eyes with water. Dilute with running water before discharging into plumbing.

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