



THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH ROBOSEP® (SECTION A) OR THE PURPLE EASYSEP® MAGNET (SECTION B).

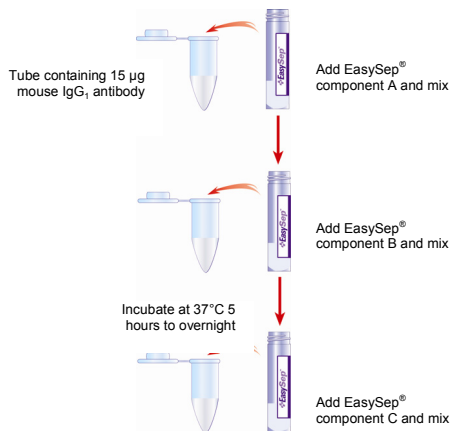
The EasySep® "Do-It-Yourself" Selection Kit contains all of the necessary components to create a specific EasySep® human positive selection cocktail when mixed with your own mouse IgG₁ monoclonal antibody. This selection cocktail, along with the EasySep® Magnetic Nanoparticles and EasySep® Magnet or RoboSep®, can then be used to positively select cells with the cell surface antigen recognized by your mouse IgG₁ monoclonal antibody (see EasySep® Labeling on reverse).

SECTION A – COCKTAIL ASSEMBLY PROCEDURE

Note: Components must be added in the proper order.

1. Add **15 µg** of your mouse IgG₁ monoclonal antibody* dissolved in your buffer of choice to a 1.5 mL polypropylene (microcentrifuge) tube. Record the volume. The total volume of antibody must not exceed 700 µL (i.e. antibody concentration must be at least 22 µg/mL).
2. Add **100 µL** of component A to the vial. Mix well.
3. Add **100 µL** of component B to the vial. Mix well. Tightly cap the vial and place it into a 37°C incubator or water bath for **5 hours or overnight**.
4. Following incubation, add **100 µL** of component C to the vial. Mix well.
5. Bring the vial to a final volume of **1.0 mL** by adding the appropriate volume of sterile phosphate buffered saline.
6. This cocktail is now ready for use in the manual EasySep® system. If using RoboSep®, transfer the cocktail to the empty vial provided. The cocktail is stable for at least one year at 2 - 8°C. Do not freeze the cocktail.

COCKTAIL ASSEMBLY PROCEDURE DIAGRAM



SECTION B – CELL SELECTION PROCEDURE

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

1. Select the appropriate RoboSep® protocol:
 - Human Do-it-Yourself Positive Selection 18099-high purity
2. 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®. When cell separation is complete, remove the tube containing the isolated cells from the magnet.

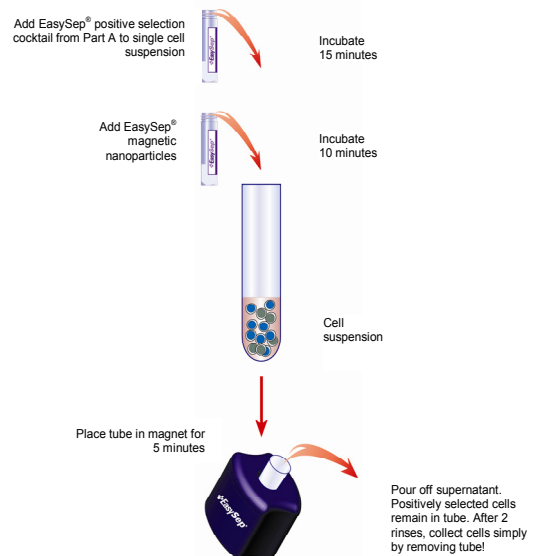
B) MANUAL EASYSEP® PROTOCOL USING THE EASYSEP® MAGNET (CATALOG #18000)

1. This procedure is used for processing **up to 5 x 10⁸ cells** per separation. Some modification may be necessary for optimal results (see Notes and Tips on reverse side). Prepare nucleated cell suspension at a concentration of 1 x 10⁸ nucleated cells/mL in recommended medium (see Notes and Tips). For rare cells, start with a concentration of 2 x 10⁸ cells/mL (see Notes and Tips - Optimizing Purity). Cells must be in a 12 x 75 mm polystyrene tube in order to properly fit into the EasySep® Magnet. Do not exceed a volume of 2.5 mL/tube. For samples containing 1 x 10⁷ cells or fewer, resuspend in 100 µL.

Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352058) are recommended.
2. Add the positive selection cocktail that has been assembled in Part A to the cell suspension at **100 µL/mL** cells (e.g. for 2 mL of cells, add 200 µL of cocktail). Mix well and incubate at room temperature (15 – 25° C) for **15 minutes**.
3. Mix EasySep® Magnetic Nanoparticles to ensure that they are in a uniform suspension by pipetting up and down more than 5 times. Vortexing is not recommended. Add nanoparticles at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of nanoparticles). Mix well and incubate at room temperature (15 – 25° C) for **10 minutes**.
4. Bring 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** (increasing separation time to 6 - 10 minutes may increase recovery - see Notes and Tips).
5. 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 inverted 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 gently pipetting up and down 2 - 3 times. Place the tube back in the magnet and set aside for **5 minutes**.
7. Repeat Steps 5 and 6, and then Step 5 once more, for a total of 3 x 5-minute separations in the magnet (for rare cells, additional rounds of separation may improve purity - see Notes and Tips). Remove tube from 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.

MANUAL EASYSEP® PROTOCOL DIAGRAM



Components:

• EasySep® "Do-It-Yourself" Component A	0.1 mL
• EasySep® "Do-It-Yourself" Component B	0.1 mL
• EasySep® "Do-It-Yourself" Component C	0.1 mL
• EasySep® Magnetic Nanoparticles	1.0 mL
• RoboSep® Vial for "Do-It-Yourself" Antibody Cocktail*	1 vial

*(not required for manual use)



POSITIVE SELECTION

REQUIRED EQUIPMENT:

EasySep® Magnet (Catalog #18000) or RoboSep® (Catalog #20000).

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep® "Do-It-Yourself" Selection Components A and B create bispecific Tetrameric Antibody Complexes (TAC) when mixed together with a mouse IgG₁ monoclonal antibody. Cells that express the surface antigen that is recognized by the mouse IgG₁ monoclonal antibody are then targeted for magnetic separation using the EasySep® Magnetic Nanoparticles and EasySep® Magnet. Component C contains an antibody against human Fc receptor to prevent nonspecific selection of monocytes and macrophages.

EASYSEP® LABELING OF HUMAN CELLS:

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

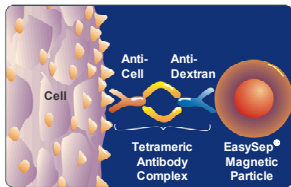


Figure 1.
Schematic Drawing of
EasySep® TAC Magnetic
Labeling of Human Cells.

NOTES AND TIPS:**PREPARING A MONONUCLEAR CELL SUSPENSION.**

Prepare a mononuclear cell suspension from whole 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 buffer 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 for optimal results. If desired cell type is to be selected from other sources/tissues, ensure that cells are in a single cell suspension for optimal results.

RECOMMENDED MEDIUM. The recommended medium is PBS with 2% FBS (Catalog #07905) and 1 mM EDTA. Medium should be Ca⁺⁺ and Mg⁺⁺ free.

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 5 and 6 an additional one to three times. Please note that recovery will decrease with each additional round of separation. Also, for rare cells (i.e. cells representing less than 5% of the initial population), increasing initial cell concentration from 1×10^8 cells/mL to 2×10^8 cells/mL may improve purity.

OPTIMIZING RECOVERY. Recovery may be improved by increasing separation time in the magnet from 5 to 10 minutes for each round. Recovery of positively selected cells is also dependent on the quality of the mouse IgG₁ antibody used in the cocktail created with the EasySep® "Do-It-Yourself" Selection Kit. 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.

ASSESSING PURITY. The mouse IgG₁ antibody used in the cocktail created with the EasySep® "Do-It-Yourself" Selection Kit may block other fluorochrome-conjugated antibodies that are used to assess purity by flow cytometry. We recommend using fluorochrome-conjugated antibody clones that are not blocked by the antibody clone used in the TAC. If this is not possible, one of the following methods can also be used to assess purity:

1. Add fluorochrome-labeled antibodies at the same time as the cocktail: Add the fluorochrome-conjugated antibody at a concentration of 0.15 - 0.4 µg/mL immediately after adding the cocktail to provide a strong detection signal without affecting separation performance. This method labels the positive cells in the entire sample.
2. Use a secondary fluorochrome-conjugated antibody, such as FITC-labeled sheep anti-mouse IgG.
3. Use alternate markers, if applicable.

COMPONENT DESCRIPTIONS:**EASYSEP® "DO-IT-YOURSELF" COMPONENT A CODE #18090**

Contains a mouse monoclonal antibody directed against dextran. This antibody has been purified from hybridoma culture supernatant by affinity chromatography using Protein A or Protein G Sepharose. The mouse monoclonal antibody subclass is IgG₁. Supplied in phosphate buffered saline.

EASYSEP® "DO-IT-YOURSELF" COMPONENT B CODE #18091

Contains a rat monoclonal antibody directed against the Fc region of Mouse IgG₁. This antibody has been purified from hybridoma culture supernatant by affinity chromatography using Protein A or Protein G Sepharose. Supplied in phosphate buffered saline.

EASYSEP® "DO-IT-YOURSELF" COMPONENT C CODE #18092C

Contains an antibody against human Fc receptor to minimize nonspecific binding of the selection reagents to monocytes and macrophages. Supplied in phosphate buffered saline.

EASYSEP® MAGNETIC NANOPARTICLES CODE #18150

A suspension of magnetic dextran iron particles in water.

ROBOSEP® VIAL FOR "DO-IT-YOURSELF" ANTIBODY COCKTAIL CODE #18093

Empty vial compatible with RoboSep® - the fully automated cell separator. If using RoboSep®, transfer the antibody cocktail assembled in Part A into this empty vial before loading into the appropriate position in the RoboSep® carousel. If not using RoboSep®, this vial can be discarded.

STABILITY AND STORAGE:**EASYSEP® "DO-IT-YOURSELF" COMPONENTS A, B AND C.**

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.

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