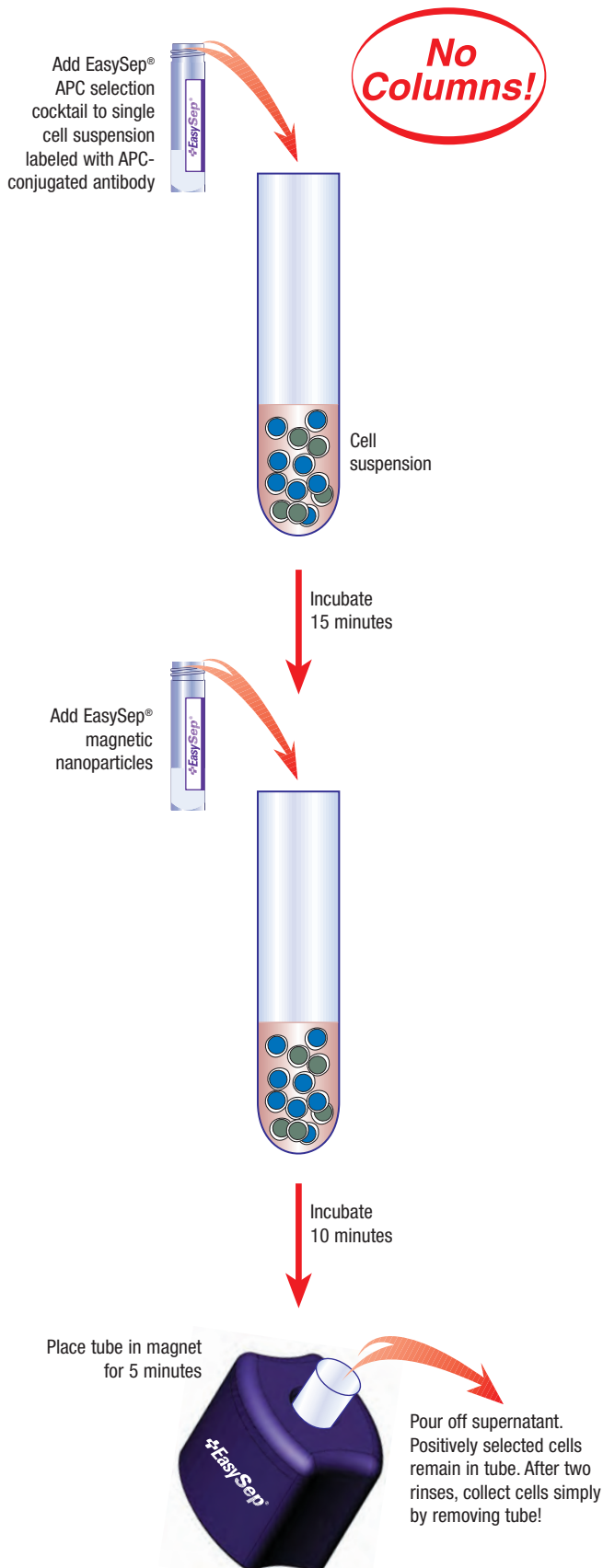


+EasySep[®] Procedure:

Note: This procedure is designed for use with the EasySep[®] Magnet (18000). If using "The Big Easy" EasySep[®] Magnet (18001), please refer to www.stemcell.com/technical/18001-PIS.pdf for additional instructions. If using RoboSep[®] - The Fully Automated Cell Separator, please follow Step 1, then select the appropriate program or refer to the RoboSep[®] user manual.



PROCEDURE

Positive Selection

And More!

Version 1.1.0

APC Selection Kit

CATALOG #18451, #18452, #18453

Some modifications to this protocol may be necessary for optimal results. See Notes and Tips on reverse side.

Note: If using RoboSep[®], APC-conjugated antibody must be transferred to the empty vial provided before loading into the carousel.

- This procedure is used for processing up to 5×10^6 cells per separation. Prepare cell suspension at a concentration of 1×10^6 nucleated cells/mL in recommended medium (See Notes and Tips). For rare cells, start with a cell concentration of 2×10^6 cells/mL (see Notes and Tips - Optimizing Purity). For samples containing 10^7 cells or fewer, resuspend in 100 μ L. **Do not exceed an initial volume of 2.5 mL per tube.** Cells must be placed in a 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.

Note: If using RoboSep[®], species-specific FcR blocking antibody should be added to the cells before they are loaded into the carousel. See Step 2 below for details.
- Add species-specific FcR blocking antibody at 100 μ L/mL for human cells (supplied with Catalog #18451) or 10 μ L/mL for mouse cells (supplied with Catalog #18452) and mix. See Product Description and Applications (reverse side) for other species (Catalog #18453).
- Add APC-conjugated antibody* at a final concentration of 0.3 - 3.0 μ g/mL. Mix well and incubate at room temperature for 15 minutes.

Note: Titrate APC-conjugated antibody for optimal purity and recovery. Cell recovery increases with increasing fluorescence intensity of the APC-labeled cells. However, excess labeling antibody can reduce purity.
- Add EasySep[®] APC Selection Cocktail at 100 μ L/mL cells (e.g. for 2 mL of cells, add 200 μ L of cocktail). Mix well and incubate at room temperature for 15 minutes.
- 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 μ L/mL cells (e.g. for 2 mL of cells, add 100 μ L of nanoparticles). Mix well and incubate at room temperature for 10 minutes.
- 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.
- 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.
- Remove the tube from the magnet and add 2.5 mL 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.
- Repeat Steps 7 and 8, and then Step 7 once more, for a total of three 5-minute separations in the magnet. (For mouse separations or low-frequency cell types, additional separations may improve purity; see Notes and Tips - Optimizing Purity). 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.

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March 2007

FOR RESEARCH USE ONLY

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| | |
|---|--|
| Catalog #18451 (Human) | For labeling 10 ⁹ total cells |
| Catalog #18452 (Mouse) | For labeling 10 ⁹ total cells |
| Catalog #18453 (Other) | For labeling 10 ⁹ total cells |
| Components: | |
| • EasySep® APC Selection Cocktail | 1.0 mL |
| • Species-Specific Blocker (Human & Mouse Only): | |
| Anti-Human CD32 (FcγRII) Blocker (#18451 Only) | 1.0 mL |
| Mouse FcR Blocker (#18452 Only) | 0.1 mL |
| • EasySep® Magnetic Nanoparticles | 1.0 mL |
| • RoboSep® Vial for Primary Conjugated Antibody (not required for manual use) | 1 vial |

REQUIRED EQUIPMENT:

EasySep® Magnet (Catalog #18000), or "The Big Easy" EasySep® Magnet (Catalog #18001), or RoboSep® (Catalog #20000).

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep® APC Selection Cocktail and EasySep® Magnetic Nanoparticles target cells that are labeled with APC-(allophycocyanin)-conjugated antibodies (not supplied) for magnetic separation. The species-specific FcR blocker (anti-CD32-Human, anti-CD16/32-Mouse) 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. These reagents are designed to positively select cells labeled with APC-conjugated antibody.

EASYSEP® LABELING OF CELLS:

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

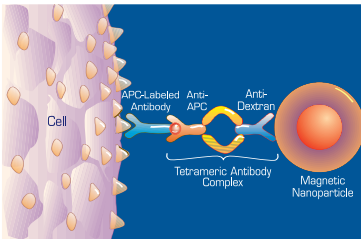


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Cells.

NOTES AND TIPS:

Preparing a 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 for optimal results. Mouse spleen and bone marrow cells should be prepared following standard procedures and filtered through a 70 µm mesh nylon strainer.

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

Assessing Purity. Since the positively selected cells have already been labeled by the APC-conjugated antibody, their purity can be assessed by flow cytometry without additional labeling.

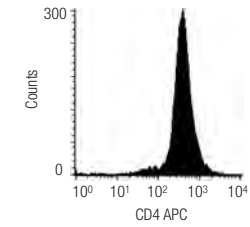
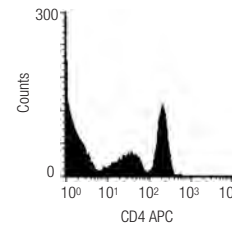
Optimizing Purity. For samples with a desired cell starting frequency of less than 10 - 15%, additional separation rounds will likely improve purity. If desired, repeat Steps 7 and 8 an additional 1 - 3 times. Please note that recovery will decrease with each additional round of separation. When isolating 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 10⁶ cells/mL. For some cell types, decreasing the amount of APC Selection Cocktail added can increase purity while decreasing recovery. This will also reduce side scatter during subsequent flow cytometry analysis. Performance may also be improved by adding a wash step. After incubating the sample with APC-conjugated 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®.

Optimizing Recovery. Recovery of positively selected APC-labeled cells is dependent on the quality of the APC-conjugated 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 APC-conjugated antibody to ensure a significant fluorescence intensity of the target cells, as there is a strong correlation between fluorescence intensity and cell recovery. We recommend that the fluorescence intensity of the positively selected cells be at least 100-fold (2 logarithms) greater than that of the negative control for adequate 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® APC SELECTION PROFILE - HUMAN CD4

Start: 27.7% CD4⁺ Cells

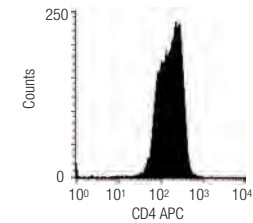
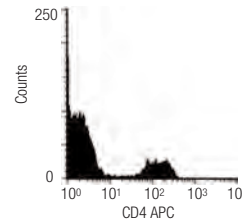
Selected: 95.5% CD4⁺ Cells



EXAMPLE: EASYSEP® APC SELECTION PROFILE - MOUSE CD4

Start: 19.2% CD4⁺ Cells

Selected: 99.4% CD4⁺ Cells



COMPONENT DESCRIPTIONS:

EasySep® APC Selection Cocktail code #18451C

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 APC (allophycocyanin) and dextran. These mouse monoclonal antibodies are of subclass 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.

Anti-Human CD32 (FcγRII) Blocker code #18520

This antibody recognizes human CD32 (FcγRII) present on monocytes, platelets, macrophages and granulocytes. Supplied in phosphate buffered saline.

EasySep® Mouse FcR Blocker code #18720

This antibody recognizes mouse CD16/CD32 (FcγRII/III Receptor) present on monocytes, macrophages and other FcR⁺ cells. Supplied in phosphate buffered saline in 0.1% BSA and 0.1% sodium azide.

EasySep® Magnetic Nanoparticles code #18150

A suspension of magnetic dextran iron particles in water.

RoboSep® Vial for Primary Conjugated Antibody code #18550

Empty vial compatible with RoboSep® - the fully automated cell separator. If using RoboSep®, transfer APC-conjugated antibody to 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® APC Selection Cocktail. Stable at 4°C for two 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 two years (human) or one 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® Magnetic Nanoparticles. Stable at 4°C for two 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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