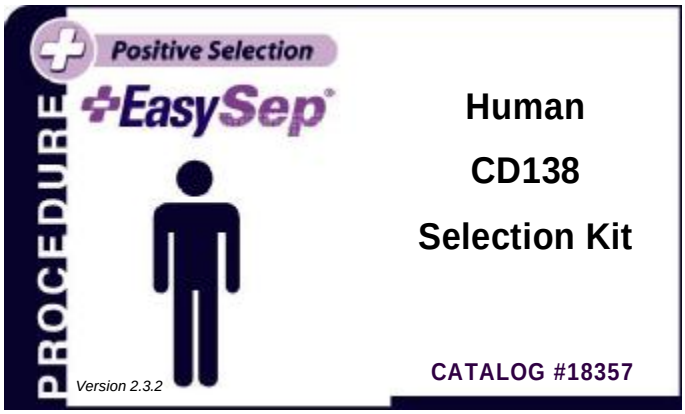


This Product Information Sheet is provided for use with RoboSep® (section A), the purple EasySep® magnet (section B) or "The Big Easy" silver EasySep® magnet (section C).

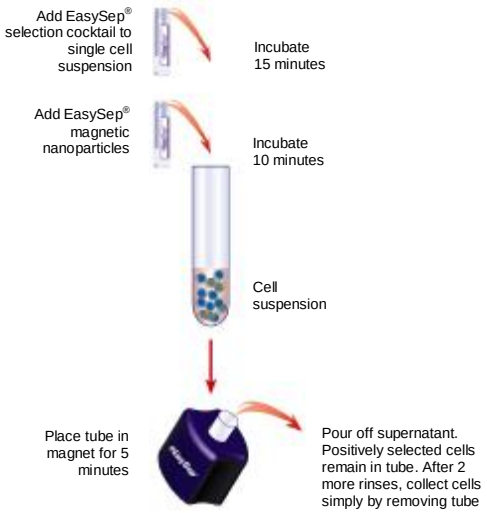
A) Fully Automated Protocol Using RoboSep® (Catalog #20000).

This procedure is used for processing **250 µL - 8.5 mL** of sample (up to 8.5 x 10^8 cells).

1. Prepare mononuclear cell suspension at a concentration of 1 x 10^8 cells/mL in RoboSep® Buffer (Catalog #20104). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep® carousel. For samples containing 2.5 x 10^7 cells or fewer, resuspend in 250 µL.
   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD Catalog #352057) are recommended.
2. Select the appropriate RoboSep® Protocol:
   - Bone marrow: Select the protocol entitled "Human CD138 Positive Selection 18357 - bone marrow".
   - PBMC: RoboSep® protocols can be optimized for high CD138$^+$ cell purity or high CD138$^+$ cell recovery. Select the protocol entitled "Human CD138 Positive Selection 18357 - high purity" or "Human CD138 Positive Selection 18357 - high recovery", as appropriate.

   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 EasySep® Magnetic Nanoparticles before loading to ensure that they are in a uniform suspension by pipetting up and down vigorously more than 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 isolated cells from the magnet. The positively selected cells are now ready for use.

Manual EasySep® Protocol Diagram



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

This procedure is used for processing **100 µL – 2.5 mL** of sample (up to 2.5 x 10^8 cells)

1. Prepare mononuclear 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. For samples containing 10^7 cells or fewer, resuspend in 100 µL.
   Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD Catalog #352058) are recommended.
2. Ÿ Bone marrow: Add EasySep® Positive Selection Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells add 100 µL of cocktail).
   Ÿ PBMC: Add EasySep® Positive 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 (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 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 (15 - 25°C) for **10** minutes.
4. 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.
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 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 samples with a CD138$^+$ starting purity of less than 10 - 15%, additional rounds of separation may be required for optimal purity. See "Notes and Tips - Optimizing Purity" on reverse. 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 – 8.5 mL** of sample (up to 8 x 10^8 cells).

1. Prepare mononuclear 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 magnet. For samples containing 2.5 x 10^7 cells or fewer, resuspend in 250 µL.
   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD Catalog #352057) are recommended.
2. Ÿ Bone marrow: Add EasySep® Positive Selection Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells add 100 µL of cocktail).
   Ÿ PBMC: Add EasySep® Positive 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 (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 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 (15 - 25°C) for **10** minutes.
4. Bring the cell suspension to a **total volume** of 5 mL (for samples containing fewer than 1 x 10^8 cells) or 10 mL (for samples containing 1 - 8.5 x 10^8 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.
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 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 5 mL (for samples containing fewer than 1 x 10^8 cells) or 10 mL (for samples containing 1 - 8.5 x 10^8 cells) 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 samples with a CD138$^+$ starting purity of less than 10 - 15%, additional rounds of separation may be required for optimal purity. See "Notes and Tips - Optimizing Purity" on reverse. 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.

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February 2008

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#28861

Printed on recycled paper.

Catalog #18357 For labeling up to 1 - 2 x 10^9 total cells
Components:
- EasySep® Human CD138 Positive Selection Cocktail 1.0 mL
- EasySep® Magnetic Nanoparticles 1.0 mL

EasySep® Positive Selection
Product Information Sheet

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

PRODUCT DESCRIPTION AND APPLICATIONS:
EasySep® Human CD138 Positive Selection Cocktail and EasySep® Magnetic Nanoparticles label $CD138^+$ cells for magnetic separation. These reagents are designed to positively select $CD138^+$ cells (cells expressing the CD138 / Syndecan-1 antigen) from fresh or previously frozen bone marrow or peripheral blood mononuclear cells.

EASYSEP® LABELING OF HUMAN CELLS:
Target cells are specifically labeled with dextran-coated magnetic nanoparticles using bispecific Tetrameric Antibody Complexes (TAC). These complexes recognize both dextran and the target cell surface antigen (Figure 1). The small size of the magnetic dextran iron particles allows for efficient binding to the TAC-labeled cells, and does not interfere with subsequent flow cytometry analysis. Magnetically labeled cells are then separated from unlabeled cells using the EasySep® procedure (reverse side).

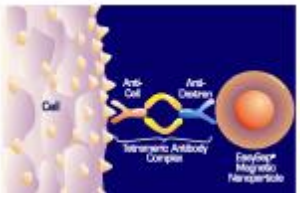


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:
Preparing a Mononuclear Cell Suspension.
- Bone marrow: Lyse red blood cells using ammonium chloride (Catalog #07800). Bone marrow mononuclear cells may also be prepared by Ficoll-Paque™ PLUS density separation (Catalog #07957).
- Peripheral blood: Prepare a peripheral blood mononuclear cell (PBMC) suspension from whole peripheral blood by Ficoll-Paque™ PLUS density separation (Catalog #07957).

For previously frozen mononuclear cells, we recommend incubating the cells with 100 µg/mL DNAse I (Catalog #07900) for at least 15 minutes at room temperature (15 - 25°C) prior to labeling and separation. Filter clumpy suspensions through a 30 µm mesh nylon strainer 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.

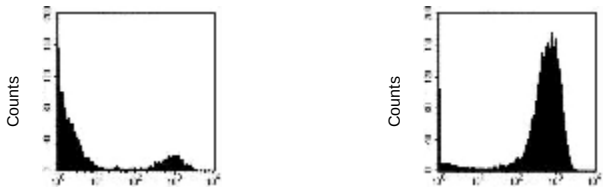
Assessing Purity. The CD138 Positive Selection Cocktail uses the anti-CD138 antibody clone B-A38. We recommend the antibody clone Mi15 to assess purity by flow cytometry. One of the following methods can also be used:

1. Stain for intracellular κ (Kappa) and λ (Lambda) light chains (e.g. procedure described by Ahmann *et al.*, Cancer Genet Cytogenet 1998, 101: 7-11). Plasma cells express either the Kappa or Lambda light chain.
2. Add PE-labeled antibodies at the same time as the cocktail: Add the fluorochrome-conjugated anti-CD138 antibody at a concentration of 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.**
3. Use alternative markers after separation: detect $CD38^+CD45^-$ cells.
4. Use a fluorochrome-conjugated secondary antibody, such as a FITC-labeled sheep anti-mouse IgG.

Optimizing Purity. For samples with a $CD138^+$ starting purity of less than 10 - 15%, additional separation rounds will likely improve purity. If desired, repeat steps 5 and 6 an additional 1 - 3 times. Note that recovery will decrease with each additional round of separation. For samples with a $CD138^+$ starting purity of less than 2%, purity of the enriched sample may be improved by starting with a cell concentration of 2 x 10^8 cells/mL.

$CD138^+$ Cell Depletion. The EasySep® CD138 Positive Selection Cocktail can also be used to deplete $CD138^+$ cells. Please refer to depletion protocol at ww.stemcell.com/technical/EasySepDepletion.pdf.

TYPICAL EASYSEP® CD138 SELECTION PROFILE:
Start: 10% $CD138^+$ Cells Selected: 91% $CD138^+$ Cells



COMPONENT DESCRIPTIONS:
EasySep® Human CD138 Positive Selection Cocktail **code #18357C.1**
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 which are directed against CD138 and dextran. The mouse monoclonal antibody subclass is IgG_1. This cocktail is supplied in phosphate buffered saline and contains an antibody against human Fc receptor. 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 #18150**
A suspension of magnetic dextran iron particles in water.

STABILITY AND STORAGE:
EasySep® Human CD138 Positive 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 (15 - 25°C), and should be refrigerated upon receipt.

EasySep® Magnetic Nanoparticles
Stable at 4°C for 2 years. Contents sterile in unopened tube. This product may be shipped at room temperature (15 - 25°C), and should be refrigerated upon receipt.

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