

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 8.5×10^8 cells).

1. Prepare a mononuclear cell suspension at a concentration of 1×10^8 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 2.5×10^7 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:

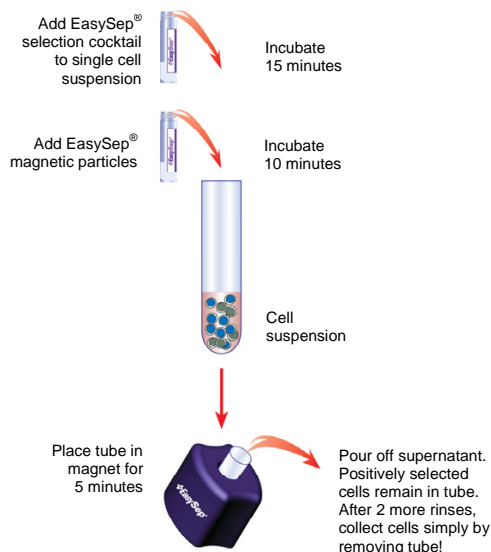
RoboSep® protocols can be optimized for high CD56⁺ cell purity or high CD56⁺ cell recovery. Select one of the protocols listed below, as appropriate.

- "Human CD56 Positive Selection 18055-high purity".
- "Human CD56 Positive Selection 18055-high recovery".

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. 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 PURPLE EASYSEP® MAGNET (CATALOG #18000).

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

1. Prepare a mononuclear cell suspension at a concentration of 1×10^8 cells/mL in the 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 purple 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.

Note: The EasySep® CD56 Positive Selection Cocktail can also be used to deplete CD56⁺ cells. Please refer to the depletion procedure at www.stemcell.com/technical/EasySepDepletion.pdf

2. 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 vigorously pipetting more than 5 times. Vortexing is not recommended. Add the 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 the cell suspension to a **total volume** of 2.5 mL by adding the 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 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 the 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. Remove tube from 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 8×10^8 cells).

1. Prepare a mononuclear cell suspension at a concentration of 1×10^8 cells/mL in the 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 silver EasySep® magnet. For samples containing 2.5×10^7 cells or fewer, resuspend in 250 µL.

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

2. 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 vigorously more than 5 times. Vortexing is not recommended. Add the 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 the cell suspension to a **total volume** of 5 mL (for $<10^8$ cells) or 10 mL (for $1 - 8 \times 10^8$ cells) by adding the 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 $<10^8$ cells) or 10 mL (for $1 - 8 \times 10^8$ cells) of the 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, then Step 5 once more, for a total of 3 x 5-minute separations in the magnet. 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.

FOR RESEARCH USE ONLY. NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES.

Components:

• EasySep [®] Human CD56 Positive Selection Cocktail	1.0 mL
• EasySep [®] Magnetic Nanoparticles	1.0 mL



POSITIVE SELECTION

REQUIRED EQUIPMENT:

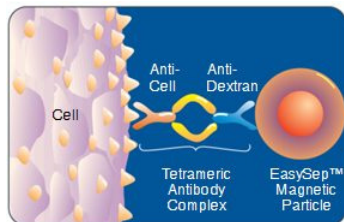
EasySep[®] Magnet (Catalog #18000), or "The Big Easy" EasySep[®] Magnet (Catalog #18001), or RoboSep[®] (Catalog #20000).

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep[®] Human CD56 Positive Selection Cocktail and EasySep[®] Magnetic Nanoparticles label CD56⁺ cells for magnetic separation. These reagents are designed to positively select CD56⁺ cells (cells expressing the CD56 antigen) from fresh or previously frozen peripheral blood mononuclear cells.

EASYSEP[®] LABELING OF HUMAN CELLS:

Unwanted cells are specifically labeled with dextran-coated magnetic nanoparticles 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:

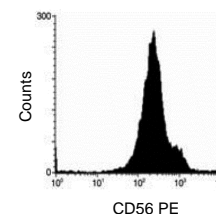
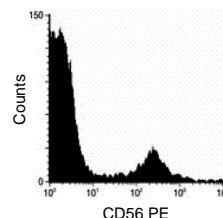
PREPARING A MONONUCLEAR CELL SUSPENSION. Prepare a mononuclear cell suspension from whole peripheral blood by Ficoll-Paque[™] density separation (Catalog #07957). For previously frozen mononuclear cells, we recommend incubating the cells with DNase I (Catalog #07900) at a concentration of 100 µg/mL for 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 RoboSep[®] Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) containing 2% Fetal Bovine Serum (FBS, Catalog #07905) and 1 mM EDTA. Media should be Ca⁺⁺ and Mg⁺⁺ free.

OPTIMIZING CELL RECOVERY. CD56⁺ cell recovery can be improved by performing a total of 2 x 5-minute separations in the magnet rather than 3, and by adding magnetic nanoparticles at an increased concentration of 100 µL/mL of cells.

ASSESSING PURITY. The EasySep[®] Human CD56 Positive Selection Cocktail uses the anti-CD56 antibody clone B159. Purity can be assessed using one of the following methods:

- Use fluorochrome-conjugated anti-CD56 clones HCD56 or CMSSB or NCAM16.2. These clones may be partially blocked but will still label CD56 positive cells.
- Use a fluorochrome-conjugated secondary antibody, such as a FITC-labeled sheep anti-mouse IgG, to detect the anti-CD56 primary antibody.

TYPICAL EASYSEP[®] CD56 POSITIVE SELECTION PROFILE:Start: 11% CD56⁺ CellsSelected: 98.1% CD56⁺ Cells

Starting with fresh peripheral blood mononuclear cells, the CD56⁺ cell content of the enriched fraction typically ranges from 86 – 98%.

COMPONENT DESCRIPTIONS:**EASYSEP[®] HUMAN CD56 POSITIVE SELECTION COCKTAIL****CODE #18055C.2**

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 CD56 and dextran. The mouse monoclonal antibody subclass is IgG₁. 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 CD56 POSITIVE SELECTION COCKTAIL**

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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