PROCEDURE

Positive Selection

+EasySep®

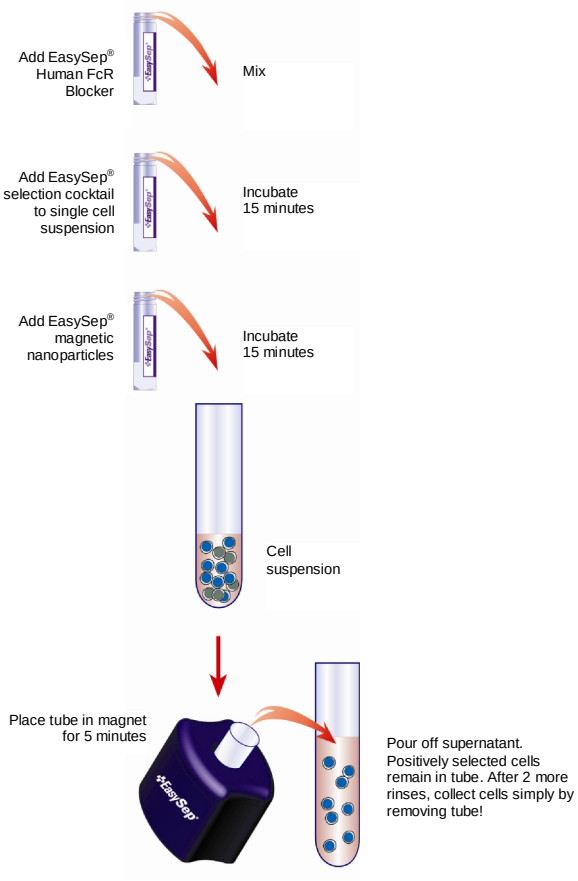
Human CD271 Selection Kit

Version 1.0.1

CATALOG #18659

This Product Information Sheet is provided for use with the purple EasySep® magnet (section A). This kit is not compatible with "The Big Easy" silver EasySep® magnet or Robosep®, the fully automated cell separator.

Manual EasySep® Protocol Diagram



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

This procedure is used for processing **500 µL - 1 mL** of sample (up to **1 x 10^8** cells).

1. Prepare nucleated single cell suspension at a concentration of 1 x 10^8 cells/mL in recommended medium (See Notes and Tips, reverse side). If using Ficolled human BM, a recommended minimum of 5 x 10^7 cells should be used per separation. Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the purple EasySep® Magnet.

   Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD Catalog #352058) are recommended.
2. Add EasySep® Human Fc receptor blocker at **25 µL/mL** cells (e.g. for 1 mL of cells, add 25 µL of cocktail). Mix well.
3. Add EasySep® Positive Selection Cocktail at **50 µL/mL** cells (e.g. for 1 mL of cells, add 50 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15** minutes.

   Note: *If starting with tissue samples other than bone marrow (e.g. cord blood or fetal liver) titration of the TAC is necessary. It is recommended to test cocktail concentrations in the range of 50 µL/mL – 150 µL/mL.*
4. 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 nanoparticles at **50 µL/mL** cells (e.g. for 1 mL of cells, add 50 µL of nanoparticles). Mix well and incubate at room temperature (15 - 25°C) for **15** minutes.
5. 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.
6. 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.***
7. 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.
8. Repeat Steps 6 and 7 once more, and then Step 6 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.

   Note: *To increase purity, it may be desirable to proceed through another round of separation (i.e. 4 x 5-minute separations in the magnet).*

STEMCELL Technologies

In North America
Tel: 1.604.877.0713
Fax:1.604.877.0704
Toll Free Tel: 1.800.667.0322
Toll Free Fax: 1.800.567.2899
e-mail: info@stemcell.com
www.stemcell.com

In the United Kingdom
Tel: +44.(0).20.7691.3561
Fax: +33.(0).4.76.18.99.63
Toll Free within United Kingdom:
Tel: 0800.731.27.14
Fax: 0800.731.27.13
e-mail: info@stemcellgb.com

In Europe
Tel: +33.(0).4.76.04.75.30
Fax: +33.(0).4.76.18.99.63
e-mail: info@stemcellfrance.com

August 2008

#29136

Catalog #18659 For labeling 2 x 10^9 total cells

Components:

• EasySep® Human CD271 Positive Selection Cocktail	1.0 mL
• EasySep® Magnetic Nanoparticles	1.0 mL
• EasySep® Human FcR Blocker	1.0 mL

EasySep® Positive Selection

Product Information Sheet

REQUIRED EQUIPMENT:

EasySep® Magnet (Catalog #18000).

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep® Human CD271 Positive Selection Cocktail and EasySep® Magnetic Nanoparticles label $CD271^+$ cells for magnetic separation. These positive selection reagents are designed to positively select $CD271^+$ cells (cells expressing the CD271 antigen) from fresh bone marrow 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 FACS analysis. Magnetically labeled cells are then separated from unlabeled cells using the EasySep® procedure (reverse side).

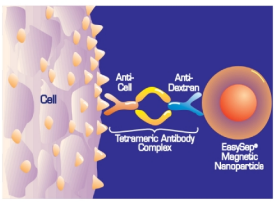


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

Preparing a Single Cell Suspension.

Bone Marrow (BM): Prepare a mononuclear cell suspension from **25 mL** human whole BM using Ficoll-Paque™ density separation (Catalog #07957).

1. Perform cell count on fresh BM sample by removing **10 µL** of BM and diluting at 1:100 with 3% glacial acetic acid. Count cells using the hemocytometer.
2. Dilute the BM with recommended medium at room temperature (15 - 25°C) to a total volume of **70 mL** (approximately 1/2.8) (e.g. dilute 25 mL of BM with 45 mL PBS for a total volume of 70 mL).
3. In three 50 mL conical tube, pour **16 mL** of Ficoll™ (Catalog #07957) into each tube. Carefully layer **23.3 mL** of diluted BM carefully on top of the Ficoll™ in each tube.
4. Centrifuge at room temperature (15 - 25°C) for **30** minutes at 300 *x g* with the break off.
5. Remove and discard the upper plasma layer without disturbing the plasma-Ficoll interface. Carefully remove and retain the mononuclear cells at the interface layer and place into a new 50 mL conical tube. Resuspend the mononuclear cells with **45 mL** of cold (2 - 8°C) recommended medium.
6. Centrifuge at 200 *x g* for **10** minutes. Discard the supernatant and repeat wash once more.
7. Discard the supernatant and resuspend to a final cell concentration of 1 x 10^8 cells/mL in cold (4°C) recommended medium. The recommended resuspension volume is **0.5** - **1.0 mL** of buffer depending on the cell number. Perform a cell count prior to EasySep® selection.
8. To determine enrichment, perform a CFU-F assay on the sample *before* and *after* CD271 selection as described in CFU-F Assay section.

Optimal Cell Number. We do not recommend the use of less than 5 x 10^7 cells per separation as this may result in sub-optimal performance.

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

Assessing Purity. The purity of enriched CD271 cells is best assayed using the CFU-F assay. Enriched populations should yield a 9 - 60 fold increase in CFU-F colonies versus unselected Ficoll-isolated BM samples. Assessment may also be done using FACS staining. However, there is a partial blockage of the CD271 epitope using this EasySep® procedure, so purity and recovery will be underestimated.

CFU-F Assay. Plate duplicates of three different cell densities in Complete MesenCult® Medium (Catalog #05411) as follows:

- Plate the *pre-enriched* mononuclear cells in T-25 cm^2 flasks at 1 x 10^6 cells/flask, 2 x 10^6 cells/flask, and 3 x 10^6 cells/flask. If using tissue culture-treated 6-well flat-bottom plates, use 2 x 10^5 cells/well, 5 x 10^5 cells/well, and 1 x 10^6 cells/well.
- Plate *post-enriched* CD271 selected BM fraction at 10-fold lower cell densities than above (e.g. plate 1 x 10^5 cells/flask, 2 x 10^5 cells/flask, and 3 x 10^5 cells/flask if using T-25 cm^2 flasks or plate 2 x 10^4 cells/well, 5 x 10^4 cells/well, and 1 x 10^5 cells/well if using 6-well flat-bottom plates).

Place the tissue culture flasks/plates into a 37°C humidified incubator with 5% CO_2 in air and >95% humidity. Score the numbers of colonies after 14 days of incubation.

Note: For more information on how to perform a CFU-F assay, please refer to the MesenCult® (Human) technical manual (Catalog #28453).

Optimizing Cell Purity. The $CD271^+$ cell purity of the enriched fraction may be improved by performing an additional round of separation in the magnet. Please note that recovery will decrease with each additional round of separation.

COMPONENT DESCRIPTIONS:

EasySep® Human CD271 Positive Selection Cocktail **code #18659C**

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 CD271 and dextran. The mouse monoclonal antibody subclass is IgG_1. 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.

Anti-Human CD32 (Fcγ RII) Blocker **code #18520**

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

STABILITY AND STORAGE:

EasySep® Human CD271 Positive Selection Cocktail

Product stable at 2 - 8°C until expiry date as indicated on label. Contents have been sterility tested. 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.

Human FcR Blocker

Product stable at 2 - 8°C until expiry date as indicated on label. Contents have been sterility tested. This product may be shipped at room temperature (15 - 25°C), and should be refrigerated upon receipt.

STEMCELL Technologies

In North America
Tel: 1.604.877.0713
Fax:1.604.877.0704
Toll Free Tel: 1.800.667.0322
Toll Free Fax: 1.800.567.2899
e-mail: info@stemcell.com
www.stemcell.com

In the United Kingdom
Tel: +44.(0).20.7691.3561
Fax: +33.(0).4.76.18.99.63
Tell Free within United Kingdom:
Tel: 0800.731.27.14
Fax: 0800.731.27.13
e-mail: info@stemcellgb.com

In Europe
Tel: +33.(0).4.76.04.75.30
Fax: +33.(0).4.76.18.99.63
e-mail: info@stemcellfrance.com