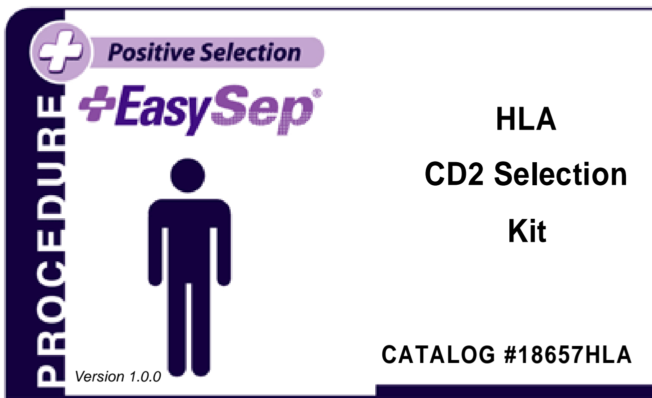


HLA CD2 Selection Kit

CATALOG #18657HLA

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

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

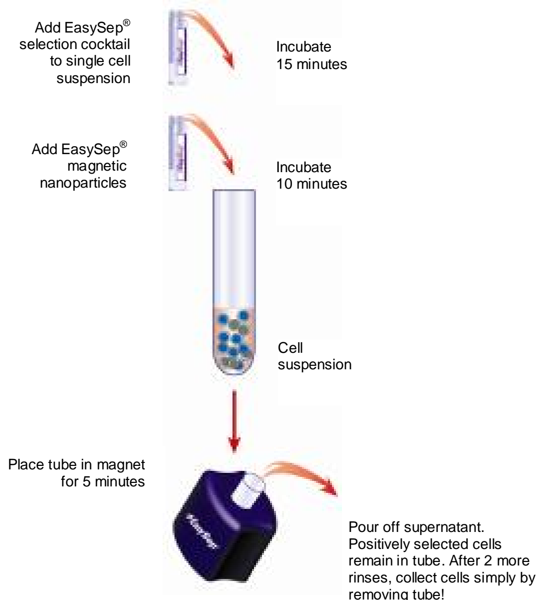
This procedure is used for processing **250 µL – 8.5 mL** of sample (up to 8.5 x 10^8 cells).

1. Prepare nucleated 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 (Becton Dickinson, Catalog #352057) are recommended.
2. Select the appropriate RoboSep® protocol:
   - For most samples, select the protocol entitled "Human CD2 Positive Selection 18657HLA".

   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. 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 and resuspend cells in an appropriate amount of desired medium. Be sure to collect any cells that may be stuck to the sides of the tube.
5. If proceeding to flow cytometric crossmatch analysis, add 200 µL of EasySep® HLA FCXM Blocking Solution (Catalog #18210HC) to the resuspended cells. 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 x 10^8 cells).

1. Prepare nucleated 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 purple EasySep® Magnet. For samples containing 10^7 cells or fewer, resuspend in 100 µL.

   Falcon™ 5 mL polystyrene round-bottom tubes (Becton Dickinson, Catalog #352058) 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 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 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 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.*
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 three 5-minute separations in the magnet. Remove the tube from the magnet and resuspend cells in an appropriate amount of desired medium. Be sure to collect any cells that may be stuck to the sides of the tube.
8. If proceeding to flow cytometric crossmatch analysis, add 200 µL of EasySep® HLA FCXM Blocking Solution (Catalog #18210HC) to the resuspended cells. The positively selected cells are now ready for use.

C) Manual EasySep® Protocol Using Silver "The Big Easy" EasySep® Magnet (Catalog #18001).

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

1. Prepare nucleated 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 silver magnet. For samples containing 2.5 x 10^7 cells or fewer, resuspend in 250 µL.

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (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 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 for **10** minutes.
4. Bring the cell suspension to a **total volume** of 5.0 mL (for <10^8 cells) or 10 mL (for 1 - 8 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 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.*
6. Remove the tube from the magnet and add 5.0 mL (for <10^8 cells) or 10 mL (for 1 - 8 x 10^8 cells) 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 three 5-minute separations in the magnet. Remove tube from magnet and resuspend cells in an appropriate amount of desired medium. Be sure to collect any cells that may be stuck to the sides of the tube.
8. If proceeding to flow cytometric crossmatch analysis, add 200 µL of EasySep® HLA FCXM Blocking Solution (Catalog #18210HC) to the resuspended cells. The positively selected cells are now ready for use.

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Catalog #18657HLA For labeling 10^9 total cells

Components:

- EasySep® HLA CD2 Positive Selection Cocktail 1.0 mL
- EasySep® Magnetic Nanoparticles 1.0 mL
- EasySep® HLA FCXM Blocking Solution 5 x 2.0 mL

REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep® HLA CD2 Positive Selection Cocktail and EasySep® Magnetic Nanoparticles label CD2⁺ cells for magnetic separation. These reagents are designed to positively select CD2⁺ cells (cells expressing the CD2 antigen) from fresh or previously frozen human peripheral blood mononuclear cells. CD2 is expressed on T cells and NK cells. Positively selected cells are compatible with flow cytometric crossmatch analysis (when used with EasySep® HLA FCXM Blocking Solution) and any other downstream assay.

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).

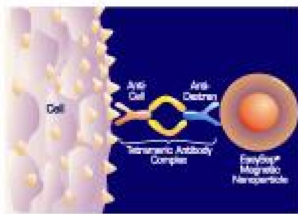


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

Preparing a Mononuclear Cell Suspension. Prepare a mononuclear cell 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 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 containing 2% FBS (Catalog #07905) and 1 mM EDTA. Medium should be Ca^{++} and Mg^{++} free.

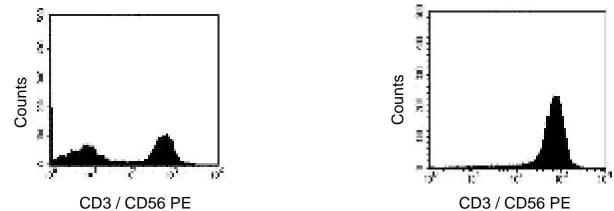
Assessing Purity. The Human CD2 Positive Selection Cocktail uses the anti-CD2 antibody clone MT910. To our knowledge, binding of this antibody blocks binding by all other anti-CD2 antibody clones used to assess purity by flow cytometry. We recommend one of the following methods to assess purity:

1. Use alternative markers after separation: detect CD3⁺ and CD56⁺ cells.
2. Use a fluorochrome-conjugated secondary antibody, such as a FITC-labeled goat anti-mouse IgG (Catalog #10210).

CD2⁺ Cell Depletion. The EasySep® HLA CD2 Positive Selection Cocktail can also be used to deplete CD2⁺ cells. Please refer to depletion procedure at www.stemcell.com/technical/EasySepDepletion.pdf.

TYPICAL EASYSEP® CD2 SELECTION PROFILE:

Start: 51.0% CD3⁺ and CD56⁺ Cells Selected: 97.7% CD3⁺ and CD56⁺ Cells



Starting with peripheral blood mononuclear cells, the CD2⁺ cell content of the enriched fraction typically ranges from 86.3 - 98.0%.

COMPONENT DESCRIPTIONS:

EasySep® HLA CD2 Positive Selection Cocktail code #18657HC
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 CD2 and dextran. The mouse monoclonal antibody subclass is IgG_1. The cocktail also includes an FcR blocker to prevent non-specific binding to monocytes. The mouse monoclonal antibody subclass of the FcR blocker is IgG_{2b}. 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.

EasySep® Magnetic Nanoparticles code #18150
A suspension of magnetic dextran iron particles in water.

EasySep® HLA FCXM Blocking Solution code #18210HC
A blocking solution required for flow cytometric crossmatch analysis following cell isolation with EasySep® or RoboSep®.

STABILITY AND STORAGE:

EasySep® HLA CD2 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, 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, and should be refrigerated upon receipt.

EasySep® HLA FCXM Blocking Solution
Stable at room temperature for 1 year. Contents sterile in unopened tube. Please note that repeated exposure to air may cause some crystallization to occur around the edge of the tube. This crystallization does not affect the performance of the blocking solution in flow cytometric crossmatch analysis.

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