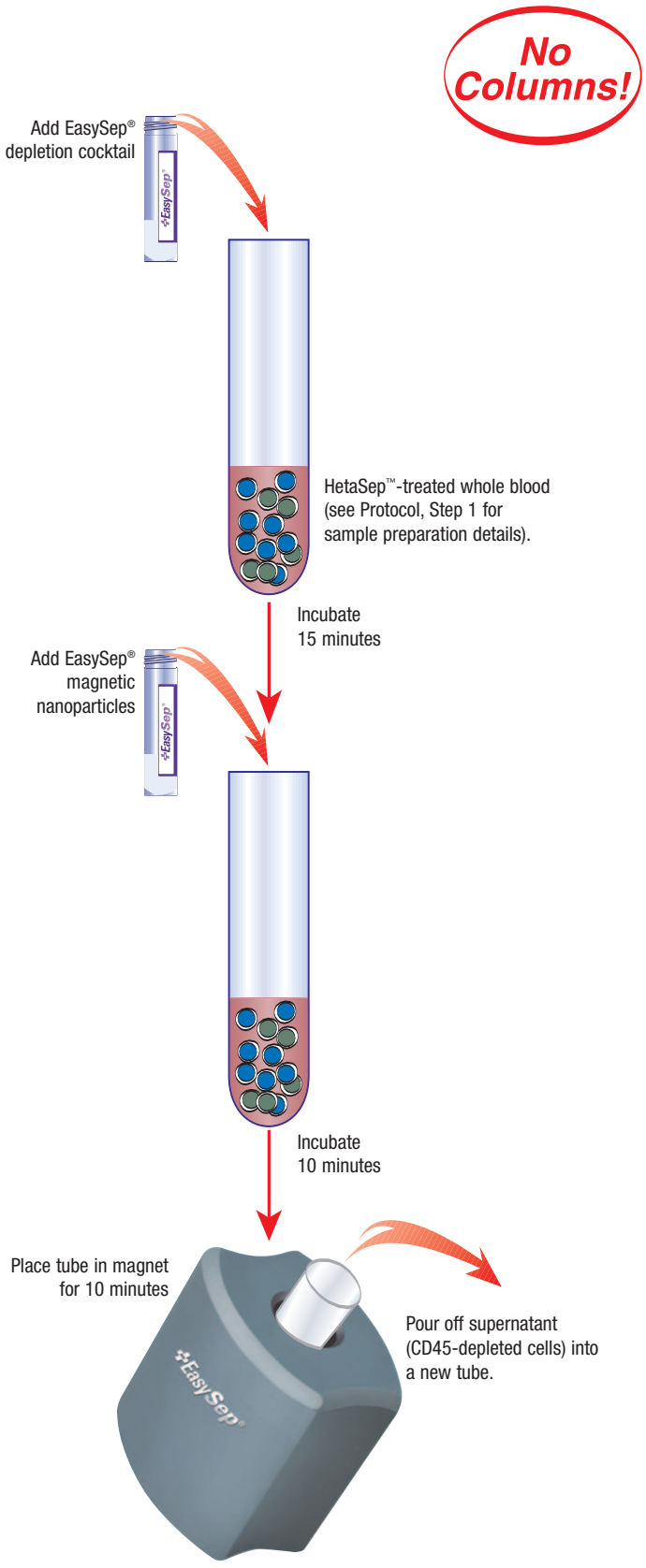
EasySep® Procedure:

Note: This procedure is designed for use with "The Big Easy" EasySep® Magnet (18001). If using RoboSep® - the Fully Automated Cell Separator, please select the appropriate program or refer to the RoboSep® user manual.



PROCEDURE

Positive Selection

EasySep®

Human Whole Blood CD45 Depletion Kit

Version 1.0.0

CATALOG #18289

1. **This procedure is used for processing up to 85 mL of whole blood per separation.** Collect whole blood in a heparinized blood collection tube or bag. Add HetaSep™ at a ratio of 1 part HetaSep™ to 5 parts whole blood.

   • For large samples, Falcon™ 50 mL polypropylene tubes *(Becton Dickinson, Catalog #352070)* are recommended.

   • For smaller samples, Falcon™ 14 mL polystyrene tubes *(Becton Dickinson, Catalog #352057)* are recommended.

   Please note that this procedure will give optimal results when tubes are at least 80% full. Nucleated cell recovery can decrease when using tubes less than half full due to shorter sedimentation distance.

   Mix well and centrifuge at 50 x *g* for 5 minutes with the brake off. Allow cells to sit for 5 minutes. Do not allow to sit for more than 5 minutes, as the leukocytes will begin to settle into the interface.

   Please note that older blood samples will settle more slowly; a 5 minute spin at 200 x *g* with the brake off may be required to achieve full separation.

   Remove plasma layer (little or no RBCs) and dilute 1:1 with recommended medium (see Notes and Tips). Centrifuge at 200 x *g* for 10 minutes.

   Remove supernatant and resuspend cells in 1/10th of the starting volume in a 14 mL polystyrene tube. Cells must be placed in a 14 mL polystyrene tube to properly fit in the EasySep® magnet. **Do not exceed a volume of 8.5 mL.**

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

2. Add EasySep® Whole Blood Depletion Cocktail at 100 µL/mL of sample mixture (e.g. for 2 mL of sample mixture, 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 100 µL/mL of sample mixture (e.g. for 2 mL of sample mixture, add 200 µL of particles). Mix well and incubate at room temperature for 10 minutes.
4. If total volume is less than 2.5 mL, add recommended medium to 5 mL, otherwise add recommended medium to 10 mL. 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 ten minutes.
5. Pick up "The Big Easy" EasySep® Magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant fraction into a new tube. The magnetically labeled cells will remain inside the tube, held by the magnetic field of the 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. The cells in the supernatant fraction are depleted of CD45 expressing cells and are now ready for use.

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August 2006

FOR RESEARCH USE ONLY

#29037

Printed on recycled paper.

Catalog #18289 For labeling up to 100 mL whole blood

Components:

• EasySep® Human Whole Blood CD45 Depletion Cocktail	1.0 mL
• EasySep® Magnetic Nanoparticles	1.0 mL
• HetaSep™	20 mL

EasySep® Positive Selection

Product Information Sheet

REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep® Human Whole Blood CD45 Depletion Cocktail and EasySep® Magnetic Nanoparticles label $CD45^+$ cells for magnetic separation. These reagents are designed to deplete $CD45^+$ cells (cells expressing the CD45 antigen) from fresh whole blood. The CD45 antigen is expressed on all human leukocytes.

EASYSEP® LABELING OF HUMAN CELLS:

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 cytometric analysis. Magnetically labeled cells are then separated from unlabeled cells using the EasySep® procedure (reverse side).

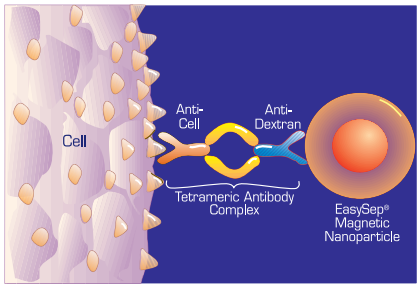


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

Recovery and Lysis. This no-lysis kit is designed for mid-range recovery of $CD45^-$ cells, along with between 2 and 4 log depletion of $CD45^+$ cells. If higher recovery of $CD45^-$ cells is desired, particles and cocktail may be titrated to achieve this. If lysis of red blood cells is acceptable, then add 10 parts ammonium chloride (Catalog #07850) to one part whole blood. Incubate for 15 minutes on ice, centrifuge at 200 x *g* for 10 minutes, discard supernatant, resuspend in recommended medium at 1/10th of the starting volume and proceed with step 2.

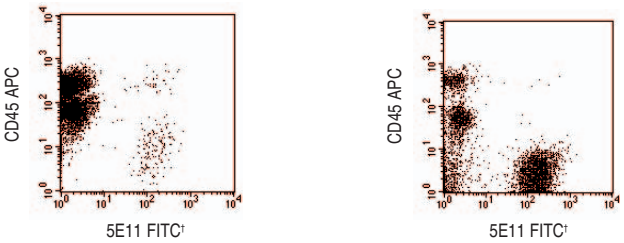
Recommended Medium. The recommended medium is PBS containing 2% FBS (Catalog #07905) and 1 mM EDTA. Media should be Ca^{++} and Mg^{++} free.

Assessing Purity. Purity of the $CD45^-$ cell fraction may be assessed by flow cytometry after staining with fluorochrome-conjugated anti-CD45 (e.g. FITC anti-CD45, Catalog #10417) and anti-Glycophorin A (e.g. PE anti-Glycophorin A, Catalog #10523).

TYPICAL EASYSEP® CD45 DEPLETION PROFILE:*

Start: 98.3% $CD45^+$ Cells and 1.7% CAMA cells (seeded into whole blood and processed with HetaSep).

Depleted: 2 log depletion of $CD45^+$ Cells



Starting with whole blood, the depletion of $CD45^+$ cells ranges from 2-4 log.

*Plots were gated on Glycophorin A negative cells to exclude residual red blood cells.
†5E11 is an antibody against an epithelial cell surface antigen expressed on CAMA cells.

COMPONENT DESCRIPTIONS:

EasySep® Human Whole Blood CD45 Depletion Cocktail **code #18289C**

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 CD45 and dextran. The mouse monoclonal antibody subclass is IgG_1. 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.

HetaSep™ **code #07806**

Hetastarch solution used to separate nucleated cells from erythrocytes in whole blood.

STABILITY AND STORAGE:

EasySep® Human Whole Blood CD45 Depletion 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.

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.

HetaSep™. Stable at 4°C for at least two years. Contents sterile in unopened bottle. This product may be shipped at room temperature, and should be refrigerated upon receipt.

See Material Safety Data Sheet for more information.

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