PROCEDURE

EasySep™

NEGATIVE SELECTION

# HLA B CELL ENRICHMENT: COMPLETE PROCESSING KIT FOR WHOLE BLOOD

CATALOG #19954HLA

V 1.4.0

STEMCELL TECHNOLOGIES

**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).**

If using other EasySep™ Magnets, please visit www.stemcell.com to download the magnet-specific Product Information Sheet or contact Technical Support at techsupport@stemcell.com.

## A) FULLY AUTOMATED PROTOCOL USING ROBOSEP™ (CATALOG #20000).

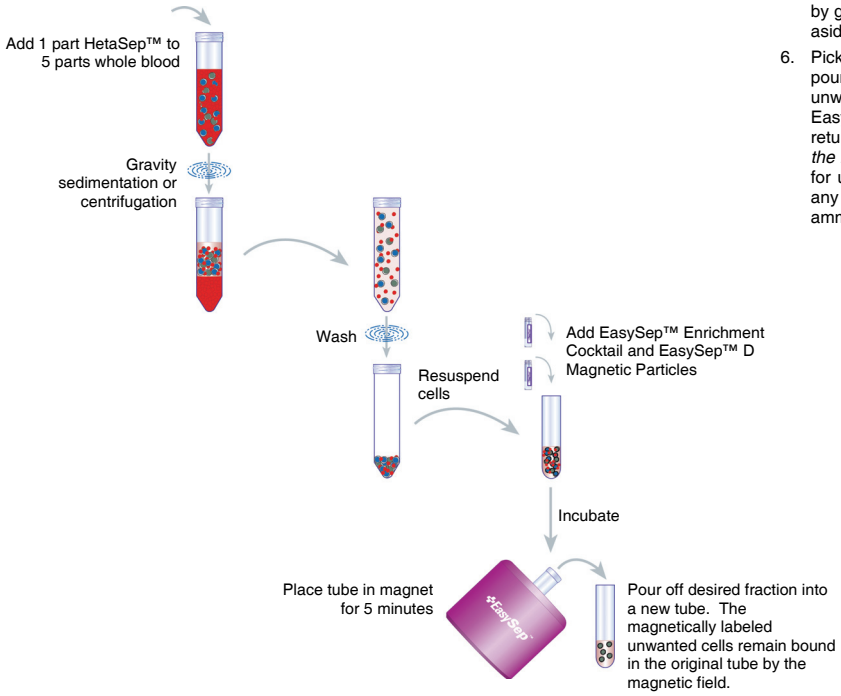
This procedure is used for processing **250 µL - 5 mL** of sample (equivalent to 2.5 mL and 50 mL of whole blood).

1. Prepare a nucleated cell suspension in RoboSep™ Buffer (Catalog #20104) using HetaSep™ (Catalog #07806: 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 RoboSep™ carousel.

   *Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352057) are recommended.*
2. Select the appropriate RoboSep™ protocol:
   - Human B Cell Negative Selection from WB 19954HLA

   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. **Vortex the EasySep™ D Magnetic Particles for 30 seconds before loading. Ensure that the particles are in a uniform suspension with no visible aggregates.** 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 enriched cells in the 50 mL tube located to the left of the tip rack. The enriched cells are now ready for use. The cells are immediately compatible with flow cytometric crossmatch analysis and any other downstream application. Any residual red blood cells can be removed by lysis using ammonium chloride (see protocol for Catalog #07800/07850) if desired.

## MANUAL EASYSEP™ PROTOCOL DIAGRAM




## B) MANUAL EASYSEP™ PROTOCOL USING THE PURPLE EASYSEP™ MAGNET (CATALOG #18000).

This procedure is used for processing **250 µL - 2 mL** of sample (equivalent to 2.5 mL and 20 mL of whole blood).

1. Prepare a nucleated cell suspension in the recommended medium using HetaSep™ (Catalog #07806: 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.

   *Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352058) are recommended.*
2. Add the EasySep™ HLA B Cell Enrichment Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.
3. Vortex the EasySep™ D Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
4. Add the EasySep™ D Magnetic Particles at **200 µL/mL cells** (e.g. for 2 mL of cells, add 400 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **5 minutes**.
5. Bring the cell suspension up 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 EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired fraction into a new 5 mL polystyrene tube. The magnetically labeled unwanted cells will remain bound inside the original 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.* The negatively selected, enriched cells in the new tube are now ready for use. The cells are immediately compatible with flow cytometric crossmatch analysis and any other downstream application. Any residual red blood cells can be removed by lysis using ammonium chloride (see protocol for Catalog #07800/07850) if desired.

## 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 (equivalent to 2.5 mL and 80 mL of whole blood).

1. Prepare a nucleated cell suspension in the recommended medium using HetaSep™ (Catalog #07806: 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.

   *Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352057) are recommended.*
2. Add the EasySep™ HLA B Cell Enrichment Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.
3. Vortex the EasySep™ D Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
4. Add the EasySep™ D Magnetic Particles at **200 µL/ mL cells** (e.g. for 2 mL of cells, add 400 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **5 minutes**.
5. Bring the cell suspension up to a **total volume** of **5 mL** (for samples of up to 5 mL) or **10 mL** (for samples of 5 - 8 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 EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired fraction into a new 14 mL polystyrene tube. The magnetically labeled unwanted cells will remain bound inside the original 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.* The negatively selected, enriched cells in the new tube are now ready for use. The cells are immediately compatible with flow cytometric crossmatch analysis and any other downstream application. Any residual red blood cells can be removed by lysis using ammonium chloride (see protocol for Catalog #07800/07850) if desired.



IN NORTH AMERICA TOLL-FREE T. 1 800 667 0322 TOLL-FREE F. 1 800 567 2899 T. +1 604 877 0713 F. +1 604 877 0704 E. INFO@STEMCELL.COM
IN EUROPE TOLL-FREE T. 00 800 7836 2355 TOLL-FREE F. 00 800 7836 2300 E. INFO.EU@STEMCELL.COM
IN AUSTRALIA TOLL-FREE T. 1 800 060 350 F. +61 (03) 9338 4320 E. INFO.AUS@STEMCELL.COM
IN SINGAPORE T. +(65) 6776 7754 F. +(65) 6776 7114 E. INFO.SG@STEMCELL.COM

CATALOG #19954HLA — For processing 200mL of whole blood

Components:

- EasySep™ HLA B Cell Enrichment Cocktail — 1.0 mL
- EasySep™ D Magnetic Particles — 4 x 1.0 mL
- HetaSep™ — 2 x 20 mL

EasySep™ NEGATIVE SELECTION

## REQUIRED EQUIPMENT:

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

## PRODUCT DESCRIPTION AND APPLICATIONS:

The EasySep™ HLA B Cell Enrichment Cocktail and EasySep™ D Magnetic Particles label non-B cells for magnetic separation. These reagents are designed to enrich B cells from HetaSep™-treated whole blood by depletion of non-B 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).

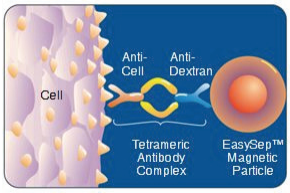



**Figure 1.**
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Human Cells.

## NOTES AND TIPS:

### PREPARING A NUCLEATED CELL SUSPENSION

1. Collect whole blood in a blood collection tube containing heparin or ACD. Using EDTA as an anticoagulant is not recommended.
2. Based on blood sample volume, select the appropriate size of tube according to Table 1. Add 1 part HetaSep™ (Catalog #07806) to 5 parts blood and mix well.
3. a. Place sample at room temperature (15 - 25°C) or in a 37°C incubator and allow to settle until the red blood cell interface is at approximately 50% of the total volume.

**OR**

b. Centrifuge the sample at room temperature (15 - 25°C) at 90 *x g* with the brake off according to Table 2. Remove sample from centrifuge and allow to sit undisturbed at room temperature (15 - 25°C) for **10 minutes**. This will allow further sedimentation of the red blood cells and will improve nucleated cell recovery.

4. Harvest the supernatant containing the nucleated cells.
5. Top up the harvested fraction with fresh buffer and centrifuge at room temperature (15 - 25°C) at 120 x *g* for **10 minutes** with the brake off.
6. Carefully remove the supernatant.

*Optional:* Lyse remaining RBCs with Ammonium Chloride Solution (see protocol for Catalog #07800/07850).

8. Resuspend cells in the recommended medium at 1/10$^{th}$ of the original starting volume (e.g. resuspend the cells recovered from 10 mL of whole blood in 1 mL of recommended medium).

**TABLE 1. TUBE SIZE RECOMMENDATIONS**

| Whole Blood Volume | Recommended Tube Size |
|---|---|
| 1 – 4 mL | Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352058) |
| 5 – 10 mL | Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352057) or 15 mL polyethylene terephthalate (PET) conical tubes (Corning, Catalog #430053) |

**TABLE 2. CENTRIFUGE TIMES BASED ON SAMPLE AGE**

| Start Volume Blood* | Tube Size | Spin Time for fresh blood (minutes) | Spin Time for 24 hour old blood (minutes) | Spin Time for 48 hour old blood (minutes) |
|---|---|---|---|---|
| 2 mL | 5 mL | 1 | 1 | 2 |
| 3 mL | 5 mL | 1 | 1 | 4 |
| 4 mL | 5 mL | 2 | 2 | 5 |
| 10 mL | 14 mL | 5 | 5 | 7 |

* Start Volume Blood refers to volume of blood before HetaSep™ addition.

**RECOMMENDED MEDIUM.** The recommended medium is RoboSep™ Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) + 2% FBS (Catalog #07905) with 1 mM EDTA. Medium should be $Ca^{++}$ and $Mg^{++}$ free.

**ASSESSING PURITY.** Purity of B cells can be measured by flow cytometry after staining with a fluorochrome-conjugated anti-CD19 antibody (e.g. PE anti-CD19, Catalog #10509).

## TYPICAL EASYSEP™ HLA B CELL ENRICHMENT PROFILE:

Start: 3.8% $CD19^+$ Cells — Enriched: 99.7% $CD19^+$ Cells


Starting with HetaSep™-treated whole blood, the $CD19^+$ cell content of the enriched fraction typically ranges from 81.5 - 99.7% (gated on $CD45^+$ cells).

Starting with 0.5 mL of HetaSep™-treated whole blood (equivalent to 5 mL of whole blood), 1.2 - 9.1 x $10^5$ $CD19^+$ cells are typically recovered.

## COMPONENT DESCRIPTIONS:

**EASYSEP™ HLA B CELL ENRICHMENT COCKTAIL** — **CODE #19954HC**

This cocktail contains a combination of monoclonal antibodies bound in bispecific Tetrameric Antibody Complexes (TAC) which are directed against cell surface antigens on human blood cells (CD2, CD3, CD14, CD16, CD33, CD36, CD41, CD43, CD56, CD66b, glycophorin A) 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™ D MAGNETIC PARTICLES** — **CODE #19250H**

A suspension of magnetic dextran iron particles in TRIS buffer.

**HETASEP™** — **CODE #07806**

Hetastarch solution used for the separation of nucleated cells from red blood cells in peripheral blood and cord blood.

## STABILITY AND STORAGE:

**EASYSEP™ HLA B CELL ENRICHMENT COCKTAIL**

**EASYSEP™ D MAGNETIC PARTICLES**

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.

**HETASEP™**

Product stable at room temperature (15 - 25°C) until expiry date as indicated on label. Contents have been sterility tested.



IN NORTH AMERICA TOLL-FREE T. 1 800 667 0322 TOLL-FREE F. 1 800 567 2899 T. +1 604 877 0713 F. +1 604 877 0704 E. INFO@STEMCELL.COM
IN EUROPE TOLL-FREE T. 00 800 7836 2355 TOLL-FREE F. 00 800 7836 2300 E. INFO.EU@STEMCELL.COM
IN AUSTRALIA TOLL-FREE T. 1 800 060 350 F. +61 (03) 9338 4320 E. INFO.AUS@STEMCELL.COM
IN SINGAPORE T. +(65) 6776 7754 F. +(65) 6776 7114 E. INFO.SG@STEMCELL.COM

V1.4.0
#29104