PROCEDURE

RosetteSep®
EasySep®

HUMAN
CORD BLOOD
CD34
POSITIVE SELECTION
KIT

CATALOG #18096

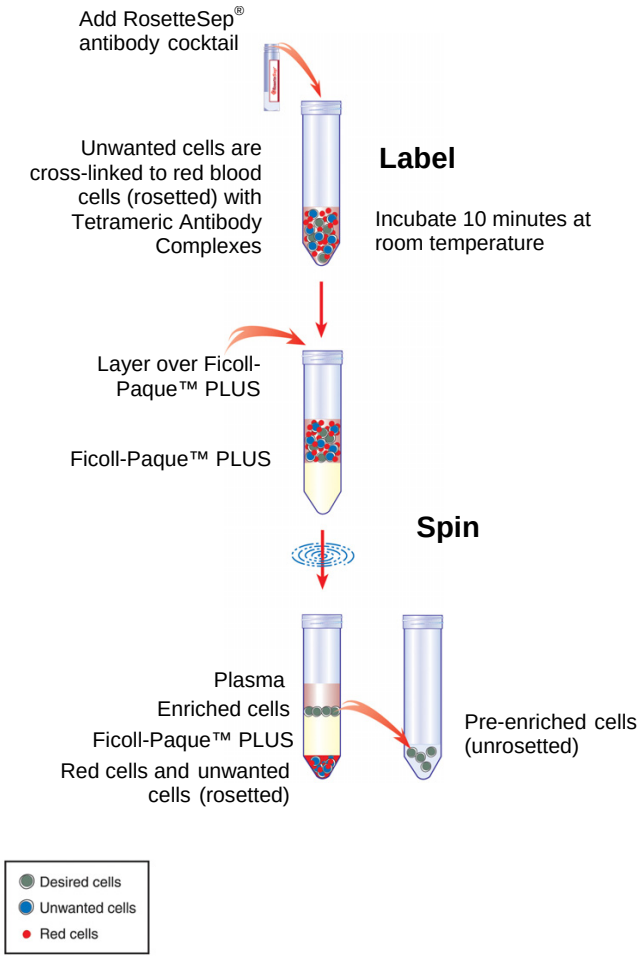
STEMCELL TECHNOLOGIES

V 2.0.1

PRODUCT DESCRIPTION

The EasySep® Human Cord Blood CD34 Positive Selection Kit (Catalog #18096) is a two-step cell isolation kit. $CD34^+$ cells are first pre-enriched from fresh whole cord blood using the RosetteSep® Human Cord Blood CD34 Pre-Enrichment Cocktail (Catalog # 15631) by negative selection (Section A, page 1). Following pre-enrichment, cells are positively selected using EasySep® Human CD34 Positive Selection Kit (Catalog #18096) (Section B, page 3).

ROSETTESEP® PROTOCOL DIAGRAM



SECTION A:

RosetteSep® Human Cord Blood CD34 Pre-Enrichment Cocktail Protocol

Ensure that cord blood sample, recommended medium (see Notes and Tips, page 2), Ficoll-Paque™ PLUS (Catalog #07957), and centrifuge are all at room temperature (15 - 25°C).

1. Add RosetteSep® Human Cord Blood CD34 Pre-Enrichment Cocktail at **5 µL/mL** of cord blood (e.g. for 20 mL of cord blood, add 100 µL of cocktail). Mix well.
2. Incubate **10 minutes** at room temperature (15 - 25°C).
3. Dilute sample with an equal volume of the recommended medium and mix gently.
4. Layer the diluted sample on top of the Ficoll-Paque™ PLUS

   OR

   Layer the Ficoll-Paque™ PLUS underneath the diluted sample.

   Be careful to minimize mixing of Ficoll-Paque™ PLUS and sample.

   See Table 1 below for volume recommendations. With 50 mL centrifuge tubes, we suggest using a minimum of 15 mL Ficoll-Paque™ PLUS to make it easier to remove the enriched cell layer.

Table 1: Recommended Volumes and Tube Sizes

Whole Blood (mL)	Recommended Medium (mL)	Density Medium (mL)	Tube Size (mL)
1	1	1.5	5
2	2	3	14
3	3	3	14
4	4	4	14
5	5	15	50
10	10	15	50
15	15	15	50

5. Centrifuge for **30 minutes** at 400 x *g* (See Notes and Tips, page 2) at room temperature (15 - 25°C), with the brake off.
6. Remove the pre-enriched cells from the Ficoll-Paque™ PLUS:plasma interface. Transfer to a new 50 mL tube.

   Removing the plasma layer before collecting the cells at the interface helps minimize platelet contamination. Sometimes it is difficult to see the cells at the interface. It is advisable to remove some of the Ficoll-Paque™ PLUS along with the enriched cells in order to ensure complete recovery of pre-enriched cells.
7. Wash pre-enriched cells by topping-up tube with recommended medium. Centrifuge at 300 x *g* for **10 minutes**. Pipette off and discard supernatant.
8. Repeat wash step. Centrifuge at 120 x *g* for **10 minutes** with brake off. Carefully aspirate supernatant.

   This slow spin helps to further remove platelets.
9. Resuspend pre-enriched cells at 2 x 10^8 cells/mL in recommended medium. The enriched cells are now ready for EasySep® Human Cord Blood CD34 Positive Selection Kit (Catalog #18096) (Section B, page 3).

Catalog #15631 For labeling 1000 mL of cord blood

Components:

- RosetteSep® Human Cord Blood CD34 Pre-Enrichment Cocktail 2 x 2.5 mL

RosetteSep® EasySep®

PRODUCT INFORMATION SHEET

PRODUCT DESCRIPTION & APPLICATIONS:

The RosetteSep® Cord Blood CD34 Pre-enrichment Cocktail (Catalog #15631) is designed to deplete granulocytes ($CD66b^+$ cells) thereby pre-enriching $CD34^+$ cells from fresh whole cord blood prior to EasySep® Human Cord Blood CD34 positive Selection (Catalog #18096).

ROSETTESEP® LABELING OF HUMAN CELLS:

The RosetteSep® antibody cocktail crosslinks unwanted cells in human whole blood to multiple red blood cells (RBCs), forming immunorosettes (Figure 1). This increases the density of the unwanted (rosetted) cells, such that they pellet along with the free RBCs when centrifuged over a buoyant density medium such as Ficoll-Paque™ PLUS. Desired cells are never labeled with antibody and are easily collected as a highly enriched population at the interface between the plasma and the buoyant density medium.

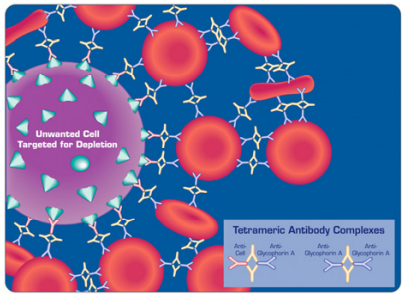


Figure 1.

Rosette of Unwanted Cells and RBCs Formed by RosetteSep® Tetrameric Antibody Complexes (TAC)

NOTES AND TIPS:

Recommended Medium. Phosphate Buffered Saline (PBS) with 2% Fetal Bovine Serum (FBS) (Catalog #07905) + 1 mM EDTA. Medium should be Ca^{++} and Mg^{++} free.

Sample Preparation. We recommend using cord blood < 24hrs old that has preferably been stored at room temperature (15 - 25°C). If there are large clumps, pass the sample through a 70 µm cell strainer.

Sample Variability. Some cord blood red cells may be less dense than in other whole blood samples, which can lead to incomplete removal of the RBCs after centrifugation. RBC aggregation may be observed at the interface following centrifugation. This does not adversely affect granulocyte depletion or subsequent EasySep® positive selection.

Density Medium. The recommended density medium is Ficoll-Paque™ PLUS (Catalog #07957). For use with HetaSep™, please contact Technical Support at techsupport@stemcell.com.

Conversion of g to RPM. To convert g to rpm, use the following formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) \times (Radius)}}$$

Where: RCF = relative centrifugal force (*g*)

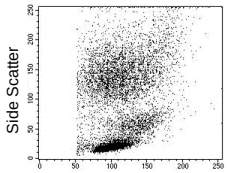
RPM = centrifuge speed in revolutions per minute

Radius = radius of rotor in cm

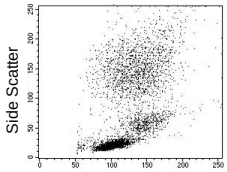
Assessing Depletion: Granulocyte depletion can be monitored by flow cytometry to evaluate depletion of high side scatter cells.

TYPICAL ROSETTESEP® GRANULOCYTE DEPLETION PROFILE:

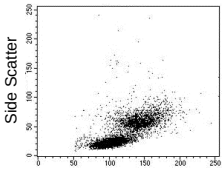
Start: 32% Granulocytes



Ficoll-Paque™ PLUS Alone: 25% Granulocytes



RosetteSep®: <1% Granulocytes



COMPONENT DESCRIPTION:

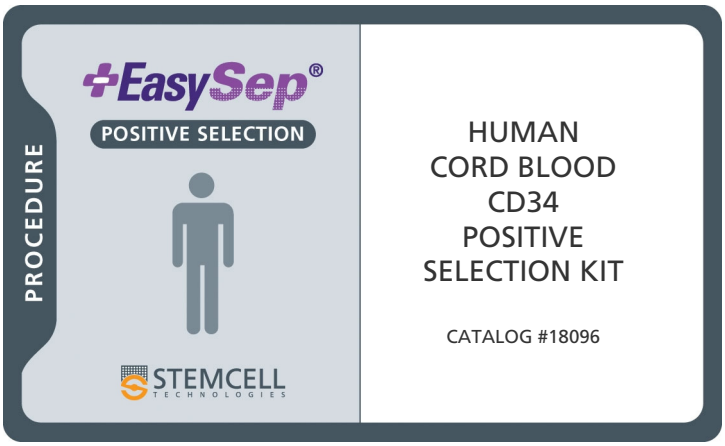
ROSETTESEP® CORD BLOOD CD34 PRE-ENRICHMENT COCKTAIL **CODE #15631C**

This cocktail contains a combination of mouse and rat monoclonal antibodies purified from mouse ascites fluid or hybridoma culture supernatant, by affinity chromatography using Protein A or Protein G Sepharose. These antibodies are bound in bispecificic TAC which are directed against cell surface antigens on human hematopoietic cells (CD66b) and glycophorin A on red blood cells. The mouse monoclonal antibody subclass is IgG_1. It should be kept in mind that this product is a biological reagent, and as such cannot be completely characterized or quantified. Some variability is unavoidable.

STABILITY AND STORAGE:

ROSETTESEP® CORD BLOOD CD34 PRE-ENRICHMENT 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.



SECTION B:

The EasySep® Human Cord Blood CD34 Positive Selection Kit is designed to select $CD34^+$ cells from cord blood samples that have been pre-enriched using the RosetteSep® Human Cord Blood CD34 Pre-Enrichment Cocktail (Catalog #15631). This procedure is compatible with the EasySep® Magnet (Catalog #18000), "The Big Easy" EasySep® Magnet (Catalog #18001) or RoboSep® (Catalog #20000).

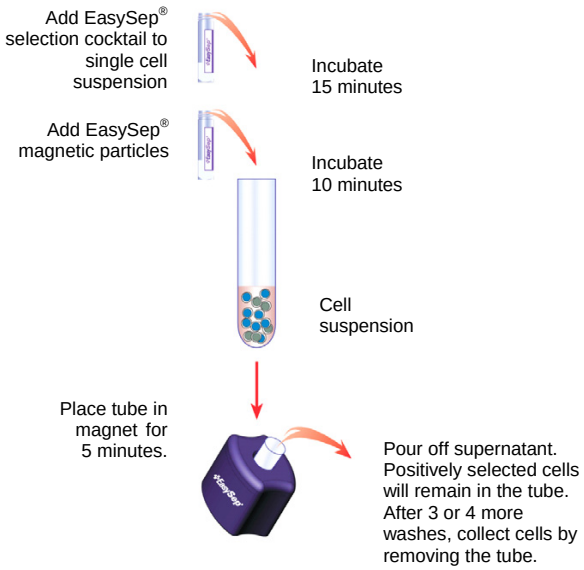
Fully Automated Protocol Using RoboSep® (Catalog #20000)

Ensure that 1X EasySep® RBC Lysis Buffer is at room temperature (15 - 25°C) (see Notes and Tips, page 4).

1. This procedure is used for processing **0.25 - 4.25 mL** (up to 8.5 x 10^8 cells) of mononuclear cell sample prepared with RosetteSep® Cord Blood CD34 Pre-Enrichment Cocktail (Catalog #15631).
2. Adjust cell concentration to 2 x 10^8 cells/mL in RoboSep® Buffer (Catalog #20104). Cells must be placed in a 14mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep® carousel.

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.
3. Add 1 part 1X EasySep® RBC Lysis Buffer (see Notes and Tips, page 4) to 1 part nucleated cell sample. Mix well.
4. Select the appropriate RoboSep® protocol:
   - Human CD34 Cord Blood Positive Selection 18096
5. If a modified RoboSep® protocol is required, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.
6. 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®.
7. When cell separation is complete, remove the tube containing the isolated cells from the magnet. Centrifuge sample at 300 x *g* for **10** minutes. Remove the supernatant and resuspend the cells in the desired medium. The positively selected cells are now ready for use.

MANUAL EASYSEP® PROTOCOL DIAGRAM



Manual Protocol Using EasySep® Magnet (Catalog #18000)

Ensure that 1X EasySep® RBC Lysis Buffer is at room temperature (15 - 25°C) (see Notes and Tips, page 4).

1. This procedure is used for processing **100 µL - 0.5 mL** (up to 1 x 10^8 cells) of mononuclear cell sample prepared with RosetteSep® Cord Blood CD34 Pre-Enrichment Cocktail 15631 (Catalog #15631).
2. Adjust cell concentration to 2 x 10^8 cells/mL in recommended medium (see Notes and Tips, page 4). Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the EasySep® Magnet.

   Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352058) are recommended.
3. Add 1 part 1X EasySep® RBC Lysis Buffer (see Notes and Tips, page 4) to 1 part mononuclear cell sample. Mix well.
4. Add EasySep® Positive Selection Cocktail at **100 µL/mL** of cell sample/lysis buffer mixture (e.g. for 1 mL of cell sample/lysis buffer mixture, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15** minutes.
5. 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 particles at **50 µL/mL** of cell sample/lysis buffer mixture (e.g. for 1 mL of cell sample/lysis buffer mixture, add 50 µL of nanoparticles). Mix well and incubate at room temperature (15 - 25°C) for **10** minutes.
6. Bring the cell suspension to a **total volume** of 2.5 mL by adding the recommended medium. Mix the cells by gently pipetting up and down 2 - 3 times. Place the tube (without cap) into the magnet. Set aside for **5** minutes.
7. 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.*
8. 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.
9. Repeat Steps 7 and 8 three times, and then Step 7 once more, for a total of 5 x 5-minute separations in the magnet. Remove the tube from the magnet and top-up tube with recommended medium to collect all cells from the side of the tube.
10. Centrifuge sample at 300 x *g* for **10** minutes. Remove supernatant and resuspend cells in desired medium. The positively selected cells are now ready for use.

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

Ensure that 1X EasySep® RBC Lysis Buffer is at room temperature (15 - 25°C) (see Notes and Tips, page 4).

1. This procedure is used for processing **125 µL - 4.25 mL** of mononuclear cell sample (up to 8.5 x 10^8 cells) prepared with RosetteSep® Cord Blood CD34 Pre-Enrichment Cocktail (Catalog #15631).
2. Adjust cell concentration to 2 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.

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.
3. Add 1 part 1X EasySep® RBC Lysis Buffer (see Notes and Tips, page 4) to 1 part sample. Mix well.
4. Add EasySep® Positive Selection Cocktail at **100 µL/mL** of cell sample/lysis buffer mixture (e.g. for 2 mL of cell sample/lysis buffer mixture, add 200 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15** minutes.
5. 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** of cell sample/ lysis buffer mixture (e.g. for 2 mL of cell sample/lysis buffer mixture, add 100 µL of nanoparticles). Mix well and incubate at room temperature (15 – 25°C) for **10** minutes.
6. Bring the cell suspension to a **total volume** of 5.0 mL (for <10^8 cells) or 10 mL (for 1 x 10^8 - 8.5 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.
7. 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.*
8. Remove the tube from the magnet and add 5.0 mL (for <10^8 cells) or 10 mL (for 1 x 10^8 - 8.5 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.
9. Repeat Steps 7 and 8 two times, and then Step 7 once more, for a total of 4 x 5-minute separations in the magnet. Remove the tube from the magnet and top-up tube with recommended medium to collect all cells from the side of the tube.
10. Centrifuge sample at 300 x *g* for 10 minutes. Remove supernatant and resuspend cells in desired medium. The positively selected cells are now ready for use.

Catalog #18096 For labeling 2 x 10^9 cells

Components:

- EasySep® Human Cord Blood CD34 Positive Selection Cocktail 2 x 1.0 mL
- EasySep® Magnetic Nanoparticles 1.0 mL
- EasySep® RBC Lysis Buffer 10X Concentrate 10 mL

RosetteSep® EasySep®

PRODUCT INFORMATION SHEET

REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION AND APPLICATIONS:

The EasySep® Human Cord Blood CD34 Positive Selection Cocktail and EasySep® Magnetic Nanoparticles label $CD34^+$ cells for magnetic separation. These positive selection reagents are designed to positively select $CD34^+$ cells (cells expressing the CD34 antigen) from fresh umbilical cord blood. If isolating $CD34^+$ cells from fresh buffy coat, we recommend using the EasySep® Whole Blood/Buffy Coat CD34 Selection Kit (Catalog #18086). If isolating $CD34^+$ cells from any other sample type, including previously frozen cord blood mononuclear cells, we recommend using the EasySep® Human CD34 Selection Kit (Catalog #18056).

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 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 target cells using the EasySep® procedure (reverse side).

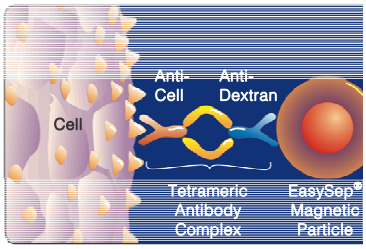


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

Preparing a Mononuclear Cell Suspension. Prepare a mononuclear cell suspension from fresh umbilical cord blood using RosetteSep® Cord Blood CD34 Pre-enrichment Cocktail (Catalog #15631C, provided). Resuspend mononuclear cells at 2 x 10^8 cells/mL in recommended medium. Use of cord blood more than 24 hours old is not recommended.

Recommended Medium. Phosphate Buffered Saline (PBS) containing 2% Fetal Bovine Serum (FBS) (Catalog #07905) and 1 mM EDTA. Medium should be Ca^{++} and Mg^{++} free.

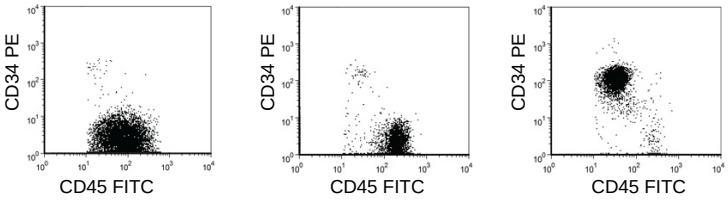
EasySep® RBC Lysis Buffer. Lysis buffer is supplied as a 10X concentrate. Prepare 1X lysis buffer at least 1 hour before use by adding 1 part 10X lysis buffer to 9 parts distilled or Type 1 water. Mix gently and completely before use at room temperature (15 - 25°C).

Assessing Purity. The CD34 Positive Selection Cocktail uses the anti-CD34 antibody clone QBend10. QBend10 is a class II antibody and may block some class I and II anti-CD34 antibody clones used to assess purity by flow cytometry. We recommend using class III anti-CD34 clones such as 8G12 (e.g. PE anti-CD34, Catalog #10513), 581, AC136, or BirmaK3 to assess purity by flow cytometry.

Note: *Flow cytometry analysis of the positively selected cells may show slightly increased side scatter relative to the start sample.*

TYPICAL FACS ANALYSIS OF HUMAN CORD BLOOD CD34 POSITIVE SELECTION KIT:

Start whole cord blood:	RosetteSep® Pre-enriched cord blood mononuclear cells:	EasySep® Human Cord Blood CD34 Positive Selection Kit:
0.5% $CD34^+$ cells	1.1 % $CD34^+$ cells	94.6% $CD34^+$ cells



Starting with fresh cord blood samples, the $CD34^+$ cell content of the enriched fraction typically ranges from 81 - 98% (gated on $CD45^+$ cells).

COMPONENT DESCRIPTION

EASYSEP® HUMAN CD34 POSITIVE SELECTION COCKTAIL — CODE #18096C

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 CD34 and dextran. The mouse monoclonal antibody subclass is IgG_1. This cocktail is supplied in phosphate buffered saline and contains purified human IgG antibodies and 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.

EASYSEP® RBC LYSIS BUFFER 10X CONCENTRATE — CODE #20110

Concentrated buffer used to lyse red blood cells prior to cell labeling and separation.

STABILITY AND STORAGE

EASYSEP® HUMAN CORD BLOOD CD34 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.

EASYSEP® RBC LYSIS BUFFER 10X CONCENTRATE

10X concentrate is stable at room temperature (15 - 25°C) for 2 years from date of manufacture as indicated on label. Store at room temperature (15 - 25°C). 1X Lysis Buffer is stable at 2 - 8°C for 3 months. Store at 2 - 8°C. Do not freeze.

STEMCELL™ TECHNOLOGIES

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