STEMSEP® PROCEDURE:

This protocol is recommended for use with commercially available magnetic positive selection columns*.

1. Prepare nucleated cell suspension at a concentration of 2 x 10^8 cells/mL in recommended medium (see Notes and Tips).
2. Add StemSep® selection cocktail at 100 µL/mL of cells (e.g. for 1 mL of cells add 100 µL of cocktail). Mix well and incubate for 10 minutes at 4 - 8°C.
3. Add CD34 PE labeled antibody if desired (see Notes and Tips).
4. Add magnetic colloid at 60 µL/mL cells (e.g. for 1 mL of cells add 60 µL of colloid). Mix well and incubate for 10 minutes at 4 - 8°C.
5. Wash cells by adding 10 - 20X the original cell suspension volume of recommended medium. Centrifuge at 300 x *g* for 8 minutes, remove supernatant and resuspend pellet to original cell volume or the maximum volume recommended for the separation column of choice.
6. Cells are ready for separation. Follow directions recommended by positive selection column manufacturer.

N.B. For increased purity, it may be necessary to perform an additional pass through a positive selection column.
*Not for use with StemSep® negative selection columns.

NOTES AND TIPS:

PREPARING A MONONUCLEAR CELL SUSPENSION. Prepare a mononuclear cell suspension from bone marrow, cord blood, or mobilized 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 to avoid blocking the separation column.

RECOMMENDED MEDIUM. The recommended medium is PBS containing 2 mM EDTA and 0.5% BSA. Medium should be Ca^{++} and Mg^{++} free. Using degassed medium reduces the chance of developing air bubbles in the column. Air bubbles cause channeling in the column, reducing the capacity of the column and potentially compromising purity.

ASSESSING PURITY. The CD34 Positive Selection Cocktail uses the anti-CD34 antibody clone QBend10. We recommend using Class III anti-CD34 clones such as: 563, HPCA-2, 581, AC136, or Birma K3 to assess purity by flow cytometry. One of the following methods can also be used to assess purity:

1. Add PE-labeled antibodies at the same time as the cocktail: Add the fluorochrome-conjugated anti-CD34 antibody (Catalog #10534) at a concentration of 0.4 µg/mL immediately after adding the cocktail to provide a strong detection signal without affecting separation performance. This method labels the positive cells in the entire sample.
2. Use alternative markers after separation: Detect $CD36^+$ cells.
3. Use a fluorochrome-conjugated secondary antibody, such as a FITC-labeled sheep anti-mouse IgG.

PROCEDURE

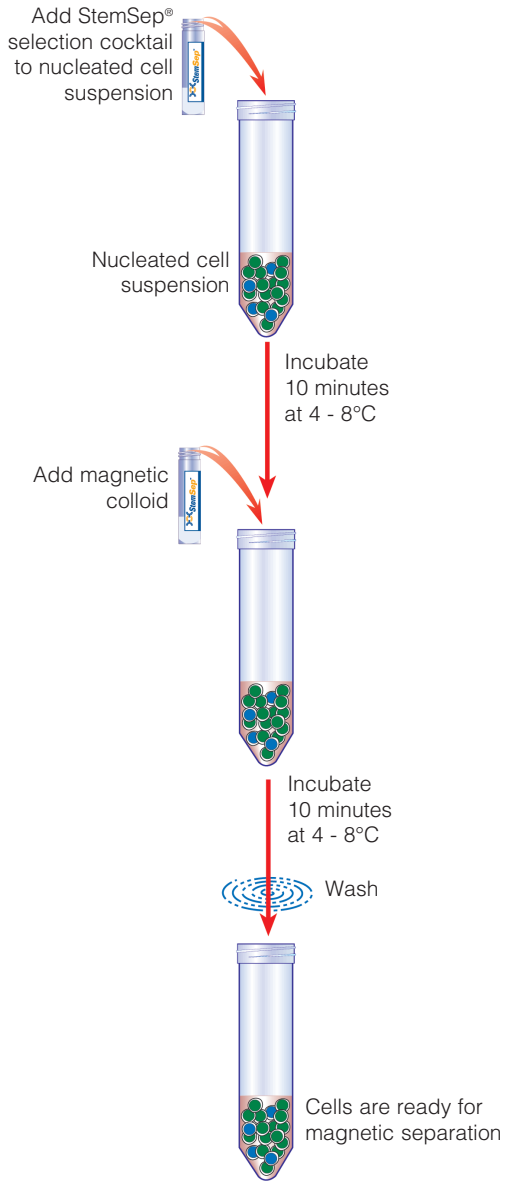
Positive Selection

StemSep®

HUMAN CD34 SELECTION COCKTAIL

VERSION 2.2.0

CATALOG #14756



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FEBRUARY 2009

FOR RESEARCH USE ONLY

#28870

CATALOG #14756 — For labeling 2 x 10^9 total cells

Components:

- StemSep® Human CD34 Positive Selection Cocktail — 1.0 mL
- StemSep® Magnetic Colloid — 1.5 mL

StemSep® Positive Selection

PRODUCT INFORMATION SHEET.

REQUIRED EQUIPMENT:

StemSep® Magnet (Catalog #11030, 11050, 11060, or 11070) or a magnet with the strength of at least 0.6 Tesla, and commercially available positive selection columns.

PRODUCT DESCRIPTION AND APPLICATIONS:

StemSep® Human CD34 Positive Selection Cocktail and StemSep® Magnetic Colloid label $CD34^+$ cells for magnetic separation. These positive selection reagents are designed to positively select $CD34^+$ cells (cells expressing the CD34 antigen) from fresh or previously frozen bone marrow, cord blood, or mobilized peripheral blood mononuclear cells.

STEMSEP® LABELING OF HUMAN CELLS:

Target cells are specifically labeled with dextran-coated magnetic colloid using bispecific tetrameric antibody complexes (TAC). These complexes recognize both dextran and the target cell surface antigen (Figure 1). The use of bispecific TAC avoids expensive and inefficient covalent coupling of antibodies to magnetic particles. The small size of the colloidal magnetic dextran iron particles allows for efficient binding to the TAC-labeled cells. Magnetically labeled cells are then separated from unlabeled cells by passing them through a magnetic separation column placed in a magnet.

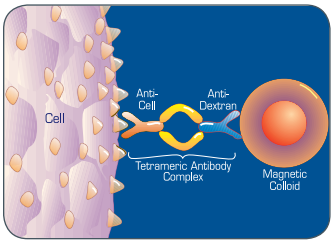
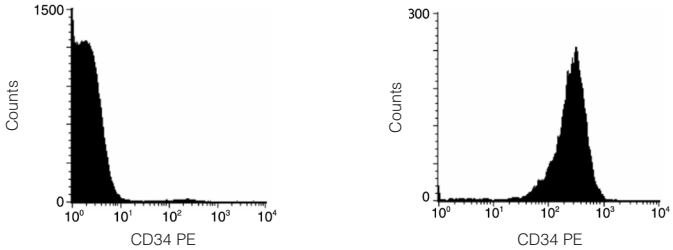


FIGURE 1.
Schematic Drawing of StemSep® TAC Magnetic Labeling of Human Cells.

TYPICAL STEMSEP® CD34 SELECTION PROFILE:

Start: 1.2% $CD34^+$ Cells — Selected: 98.8% $CD34^+$ Cells



The expected $CD34^+$ content of the enriched fraction is 97.3 ± 1.7% with expected recoveries of 68.8 ± 13.3% after 2 passes through positive selection columns.

COMPONENT DESCRIPTIONS:

STEMSEP® HUMAN CD34 POSITIVE SELECTION COCKTAIL CODE #14756C

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 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 an antibody against a 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.

STEMSEP® MAGNETIC COLLOID CODE #10051

A colloidal suspension of magnetic dextran iron particles in USP saline, pH 7.0 - 7.5.

STABILITY AND STORAGE:

STEMSEP® HUMAN CD34 POSITIVE SELECTION COCKTAIL

Stable at 2 - 8°C for 2 years. Do not freeze. This product has been sterility tested.

STEMSEP® MAGNETIC COLLOID

This product is shipped at room temperature. Once opened, stable at 2 - 8°C for 6 weeks. Stable at -20°C for 1 year. Repeated freezing and thawing is possible but not recommended. Vortex before re-freezing. This product has been sterility tested.

REFERENCES:

1. Lansdorp PM, Thomas TE: Purification and analysis of bispecific tetrameric antibody complexes. Mol Immunol 27: 659, 1990
2. Lansdorp PM, Aalberse RC, Bos R, Schutter W, Van Bruggen EFJ: Cyclic tetramolecular complexes of monoclonal antibodies: A new type of cross-linking reagent. Eur J Immunol 16: 679, 1986
3. Molday RS, MacKenzie D. Immunospecific ferromagnetic iron dextran reagents for the labelling and magnetic separation of cells. J Immunol Methods 52: 353, 1982

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