STEMSEP® ABBREVIATED PROCEDURE:

Refer to StemSep® Cell Separation Technical Manual for additional information. www.stemcell.com/technical/28416_ssmanual.pdf

1. Prepare cell suspension (see Notes and Tips) at a concentration of 5 x 10^7 cells/mL or within the acceptable range of 2 - 8 x 10^7 cells/mL in separation medium (see Notes and Tips).

2. Add StemSep® Enrichment Cocktail at 100 µL /mL cells (e.g. for 1 mL of cells add 100 µL of cocktail). Mix well.

3. Incubate on ice for 30 minutes or at room temperature for 15 minutes.

4. Add Magnetic Colloid at 60 µL/mL of cells (e.g. for 1 mL of cells add 60 µL of colloid). Mix well.

5. Incubate on ice for 30 minutes or at room temperature for 15 minutes.

6. During incubation prepare column (refer to diagrams opposite) as follows:
   a) Using Table 3 (see Notes and Tips) determine the appropriate column size based on cell number.
   Note: Do not insert column from the front of the magnet. Lower column slowly from above down into the gap of magnet.
   b) Gravity Feed - Place column in magnet and assemble. Prime with priming medium (see Notes and Tips) from the bottom up by depressing plunger of side syringe slowly.** Check for air bubbles. Proceed with 6d.
   ****Note:** 0.1" column is primed quickly.
   c) Pump Feed - Place column in magnet and assemble. Prime with priming medium (see Notes and Tips) from the bottom up at appropriate speed (see Table 1). Check for air bubbles. Proceed with 6d.
   d) Wash from the top down with appropriate volume of separation medium (see Table 2).
   Note: Do not let column run dry at anytime during priming, loading or washing of the column.

7. Load sample.

8. Wash from the top down with separation medium, collecting the sample volume plus the appropriate column wash volume as flowthrough (see Table 2). The enriched cells are now ready for use.

TABLE 1. FLOW RATES AND PUMP SETTINGS

COLUMN SIZE	PRIMING		LOADING SAMPLE AND WASHING	
	mL/min	pump setting*	mL/min	pump setting*
1.0"	2.0	10.0	5.0	27.0
0.6"	0.6	3.0	2.0	10.0
0.5"	0.3	1.5	1.0	5.0
0.3"	0.2	1.0	0.6	3.0

*Pump setting for 4-channel pump supplied by STEMCELL Technologies only.

TABLE 2. COLUMN WASH VOLUME

COLUMN SIZE	COLUMN WASH VOLUME
1.0"	90 mL
0.6"	25 mL
0.5"	15 mL
0.3"	8 mL
0.1"	1.5 mL

PROCEDURE

Negative Selection

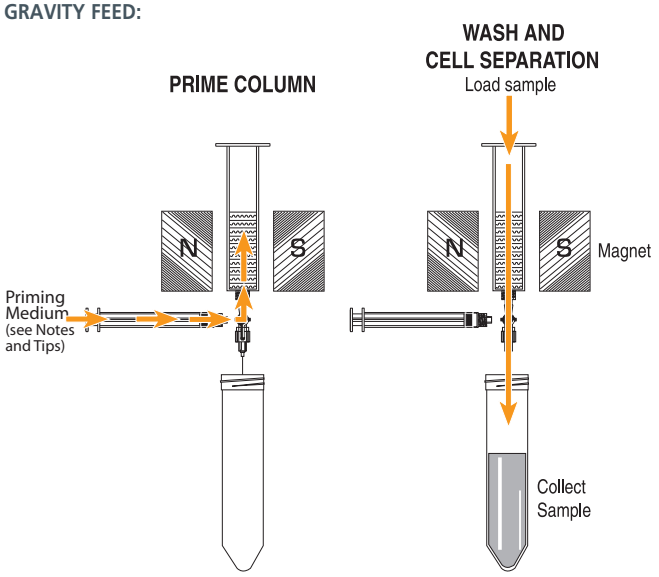
StemSep®

VERSION 2.2.1

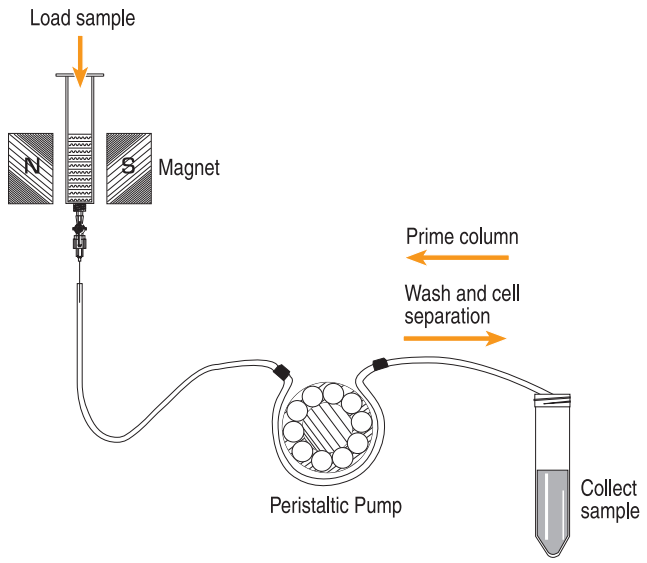
HUMAN HEMATOPOIETIC PROGENITOR CELL ENRICHMENT KIT

CATALOG #14056 (14066)

GRAVITY FEED:



PUMP FEED:



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FOR RESEARCH USE ONLY

#28876

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CATALOG #14056 (14066) For labeling 10^9 (5×10^9) total cells

Components:

• StemSep® Human Hematopoietic Progenitor Cell Enrichment Cocktail	2.0 mL (5 x 2.0 mL)
• Magnetic Colloid	1.5 mL (4 x 1.5 mL)

StemSep® Negative Selection

PRODUCT INFORMATION SHEET

REQUIRED EQUIPMENT:

StemSep® Magnet (Catalog #11030, 11050, 11060, or 11070) or a magnet with the strength of at least 0.5 Tesla, and StemSep® Negative Selection Columns (see Table 3, Notes and Tips).

PRODUCT DESCRIPTION AND APPLICATIONS:

The StemSep® Human Hematopoietic Progenitor Cell Enrichment Kit is designed to enrich hematopoietic progenitor cells from fresh or previously frozen mobilized peripheral blood, cord blood or bone marrow.

STEMSEP® LABELING OF HUMAN CELLS:

Target cells are specifically labeled with colloidal magnetic dextran iron particles using bispecific tetrameric antibody complexes (TAC). These complexes recognize both dextran and the target cell surface antigen (Figure 1). Magnetically labeled cells are then separated from unlabeled cells by passing them through a magnetic separation column placed in a magnet.

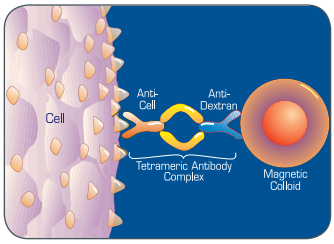


FIGURE 1.
Schematic Drawing of StemSep® TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

SAMPLE PREPARATION. Use fresh or previously frozen mobilized peripheral blood (leukaphereses), bone marrow, or cord blood. All samples must have <20 red blood cells per nucleated cell (e.g. a hematocrit of <5%). This can be achieved by ammonium chloride lysis (Catalog #07850) or Ficoll™ (see below) where appropriate.

COLUMN PREPARATION:

- Use the appropriate column size (see Table 3).
- Check all the connections during priming and washing to ensure they do not leak.
- Prime the column from the bottom up.
- Use priming medium (see below) to prime the column.
- Ensure that there are no air bubbles in the column.
- Use separation medium (see below) to wash the column. The protein present in the wash solution prevents cells from binding non-specifically to the column.
- Ensure that the column does not run dry at any time.

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 the addition of 100 mg/mL DNase (Catalog #07900) prior to labeling and separation. Excessively clumpy suspensions may be passed through a 30 - 70 µm mesh nylon strainer.

RECOMMENDED MEDIA: Using degassed media 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. Media should be Ca^{++} and Mg^{++} free.

- **PRIMING MEDIUM:** Use PBS (Catalog #37350), either at room temperature or degassed, without serum or other protein.
- **SEPARATION MEDIUM:** Use PBS + 2% FBS (Catalog #07905).

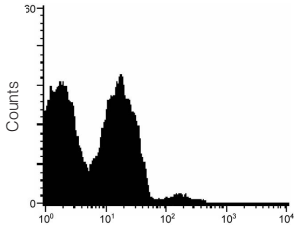
ASSESSING PURITY. Purity of hematopoietic progenitor cells can be measured using flow cytometry by staining with a fluorochrome-conjugated anti-CD34 antibody (e.g. anti-CD34 FITC, Catalog #10413).

TABLE 3. RECOMMENDED NUMBER OF HUMAN MONONUCLEAR CELLS IN THE START SUSPENSION FOR VARIOUS COLUMN SIZES

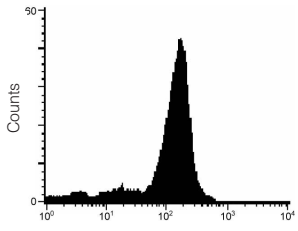
COLUMN SIZE	CATALOG # GRAVITY PUMP		COLUMN CAPACITY BASED ON CELL NUMBER	WILL FIT MAGNET SIZE
1.0"	-	12470	2×10^9 - 2.0×10^{10}	black
0.6"	12061	12062	10^8 - 1.5×10^9	green, blue, black
0.5"	12051	12052	5×10^7 - 3×10^8	green, blue, black
0.3"	12031	12032	2×10^7 - 10^8	all sizes
0.1"	12021	-	10^5 - 2×10^7	red, green

TYPICAL STEMSEP® HEMATOPOIETIC PROGENITOR CELL ENRICHMENT PROFILE:

Start: 1.6% $CD34^+$ Cells



Enriched: 82% $CD34^+$ Cells



The frequency of $CD34^+$ cells in the enriched fraction is typically: 74 - 88% (mobilized peripheral blood), 30 - 50% (bone marrow), or 45 - 61% (cord blood).

COMPONENT DESCRIPTIONS:

STEMSEP® HUMAN HEMATOPOIETIC PROGENITOR CELL ENRICHMENT COCKTAIL — CODE #14056C

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 cell surface antigens on human hematopoietic cells (CD2, CD3, CD14, CD16, CD19, CD24, 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. Supplied in phosphate buffered saline.

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 HEMATOPOIETIC PROGENITOR CELL ENRICHMENT COCKTAIL

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

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.

See Material Safety Data Sheet for more information.

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