Helpful Hints

To ensure optimal results when using StemSep®, follow these suggestions:

Reagents

- Store the reagents correctly.
  Do not freeze tetrameric antibody complex; store at 4°C.
  The magnetic colloid may be stored for up to six weeks at 4°C, or frozen at -20°C for up to one year. Repeated freezing and thawing is possible but not recommended. If freezing, vortex vigorously just prior to freezing. If particulate matter is visible when thawing, vortex and store at 4°C for 24 hours. Small particulate matter can be removed by filtering through a 0.2 µm filter.
- Use buffered salt solutions without Ca^{++} or Mg^{++} with 2 to 6% FBS.
- Add EDTA to a final concentration of 1 mM to recommended medium when enriching for adherent cells such as monocytes or dendritic cells.
- Add EDTA to a final concentration of 2 mM when enriching for circulating epithelial tumor cells.

Column Preparation

- Check all the connections during priming and washing to ensure they do not leak.
- Prime the column from the bottom up.
- Use **PBS without FBS** or other protein (serum) to prime the column.
- Ensure that there are no air bubbles in the column.
- Use PBS with FBS, or Hank's with FBS to wash the column. For enrichment of monocytes or dendritic cells, add EDTA to a final concentration of 1 mM. For enrichment of metastatic epithelial tumor cells, add EDTA to a final concentration of 2 mM.
  The protein in the wash solution blocks any protein binding sites on the mesh in the column, thus preventing cells from binding non-specifically to the column.
- Ensure that the column does not run dry at any time.

StemSep®
Procedure - Human Cells

Hematopoietic Progenitor Enrichment
Use mobilized peripheral blood (leukaphereses), bone marrow, or cord blood.

Lymphocyte (T, B, NK), Dendritic Cell, and Monocyte Enrichment
Use a mononuclear cell suspension from peripheral blood (e.g. Ficolled). Enrichment from mobilized peripheral blood (leukaphereses), bone marrow, or cord blood may require a custom cocktail.

Basophil Enrichment
Use whole blood, mobilized peripheral blood (leukaphereses) or cord blood.

Eosinophil Enrichment
Use a polymorphonuclear cell suspension (e.g. a lysed Ficoll pellet). Do not use dextran to make this suspension.

Epithelial Tumor Cell Enrichment
Use whole blood or bone marrow.

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 or Ficoll, where appropriate.

Recommended Medium: Buffered salt solutions without Ca^{++} or Mg^{++}, such as PBS, modified with 2% fetal bovine serum (FBS). The addition of EDTA is suggested to improve recovery of adherent cells (monocytes and dendritic cells: 1 mM; tumor cells: 2 mM).

Table 1. Optimum Number of Human Nucleated Cells in the Start

Column Size	Optimum # of Cells	Extended Range of Cell # for Cell Enrichment	Extended Range of Cell # for Cell Purging
1.0"	10^{10}	2×10^9 - 1.5×10^{10}	2×10^9 - 1.5×10^{10}
0.6"	5×10^8	10^8 - 1.5×10^9	10^8 - 2×10^9
0.5"	10^8	5×10^7 - 3×10^8	5×10^7 - 5×10^8
0.3"	5×10^7	2 - 8×10^7	2×10^7 - 10^8
0.1"	10^6 - 10^7	10^5 - 2×10^7	10^5 - 2×10^7

Note: When using peripheral blood mononuclear cells, column capacity may be doubled.

Abbreviated Procedure - Human Cells

Refer to the StemSep® Cell Separation Technical Manual available at www.stemcell.com for further details.

1. Resuspend cells at 5x10^7 cells/mL or within the acceptable range of 2 - 8 x 10^7 cells/mL in recommended medium (see previous page).
2. a) For monocyte and dendritic cell enrichment: Add 30 µL anti-CD32 (Fcγ RII) blocking antibody/mL cells. Incubate 10 minutes on ice. Proceed with 2c.
   b) For cocktails containing biotinylated antibodies (naïve T cell, customs): Add amount of biotinylated antibody indicated on vial. Incubate 30 minutes on ice. Wash cells and resuspend in recommended medium. Proceed with 2c.
   c) **All enrichments:** Add 100 µL cocktail/mL cells. Mix well.
3. Incubate on ice for 30 minutes or at room temperature for 15 minutes.
4. Add 60 µL of magnetic colloid/mL cells. Mix well.
5. Incubate on ice for 30 minutes or at room temperature for 15 minutes.
6. Prepare column as follows (refer to diagrams on opposite page):
   a) Pump Feed - Assemble column and prime with PBS (no protein) from the bottom up at appropriate speed (see Table 2). Check for air bubbles. Place in magnet. Proceed with 6c.

Table 2. 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.

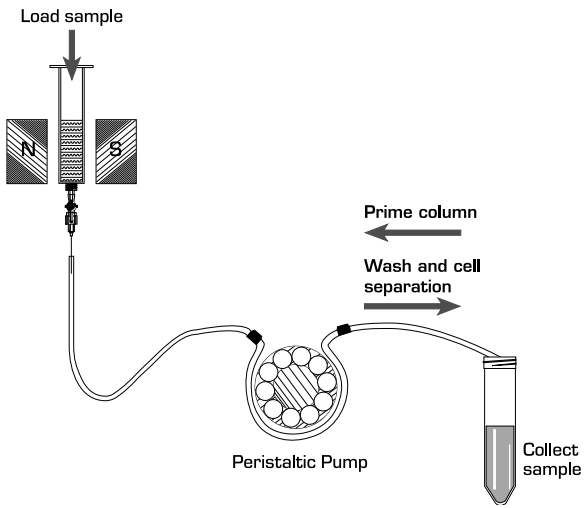
   b) Gravity Feed - Place column in magnet and assemble. Prime with PBS (no protein) from the bottom up by depressing plunger of side syringe slowly.** Check for air bubbles. Proceed with 6c.
   **Note: 0.1" column is primed quickly.
   c) Wash (top down) with 3X column volume of recommended medium (see Table 3).
7. Load sample. Wash through with recommended medium, collecting sample volume plus 3X column volume as flowthrough (see Table 3).

Table 3. 3X Column Volume

Column Size	3X Column Volume
0.6"	25 mL
0.5"	15 mL
0.3"	8 mL
0.1"	1.5 mL

Prepare Column

Pump Feed:



Gravity Feed: